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THE OSMOTIC PRESSURE OF BODY FLUIDS IN
HEALTH AND IN DISEASED CONDITIONS.

by

I. M. Harrison, B.Sc.

Summary of

Thesis submitted for the degree of Ph.D.

University of Glasgow

June 1966

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The maintenance of the normal composition and osmotic pressure of the extracellular fluid is essential for the existence of living cells. The kidney plays a major role in maintaining the homeostasis of the extracellular fluid by varying the output and the composition of the urine. The osmotic pressure of the extracellular fluids and hence that of the cells that bathe is maintained in the region of 290 mOsm. per Kg. water. The human kidney is capable of producing urine which can vary in osmolality from about 50 mOsm. per Kg. water under conditions of maximal diuresis to 1400 mOsm. per Kg. water under conditions of antidiuresis. During antidiuresis, one of the major functions of the kidney is the production of a concentrated urine. Urinary osmotic pressure reflects this concentrating ability of the kidney, and hence should bear some relationship to the functional state of the kidney.

The aim of the present study was to define more clearly the limits within which urine osmolality could be used as an index of renal concentrating ability and to establish a test of renal function based on urine osmolality which could be applied to subjects with chronic renal disease.

Urine osmolality following 14 - 15 hours dehydration was found to be a poor index of renal concentrating ability. In normal subjects under standard conditions, a very wide range of normal values was obtained. Many of the subjects could achieve a higher urine osmolality throughout the day, without long periods of dehydration.

The most significant finding in normal subjects was a linear relationship between solute excretion rate and urine flow rate in the range 14 - 75 ml. per 60 min. This range of flow rates could be attained under normal hydration

mild dehydration. At higher urine flow rates, this relationship ceased abruptly, and there was a random scatter of results. From the equation relating urine flow rate and solute excretion in the range 14 - 75 ml. per 60 min., the theoretical osmolality corresponding to a given urine flow rate was derived. The ratio observed: theoretical osmolality expressed as a percentage was taken as an index of renal concentrating ability.

This measure of concentrating ability was applied to a group of normals, a group of control patients, and a series of patients suffering from chronic renal disease. Simultaneous urea clearance tests were carried out on all subjects to assess the reliability of this test as an index of renal function. In normal subjects and control patients, both tests gave a comparable index of renal function. In the early stages of chronic renal disease there was good agreement between the tests. In the more advanced cases of chronic renal disease, there was a marked discrepancy between the two assessments, and urea clearance values were consistently lower than concentrating ability.

Changes in urine flow rate and solute excretion rate were also studied in normal subjects during a water diuresis, and were compared with a diuresis induced by urea or Frusemide. Findings in subjects with polyuria due to renal disease were compared with normal subjects. The possible application of the concentrating ability test to other clinical conditions was considered.

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AND IN DISEASED CONDITIONS.

Thesis submitted for the degree of Ph.D.
in the
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INTRODUCTION.

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OSMOTIC PRESSURE: Its Definition and Application to Biological Fluids.

The terms osmolality and osmotic pressure, now widely applied to biological fluids, were originally derived from the concept of osmosis. Osmosis was the term applied to the spontaneous flow of a solvent into a solution, when the two were separated by a semi-permeable membrane. This movement of solvent resulted in an increase in pressure within the membrane enclosed area. Osmotic pressure is that excess pressure which would have to be applied to prevent the passage of solvent into the solution via the semi-permeable membrane.

The first quantitative measurements of osmosis were carried out on sucrose solutions by Pfeffer (1877) who showed that osmotic pressure increased proportionally with temperature. At constant temperature, however, the osmotic pressure of a solution was almost directly proportional to its concentration. Osmotic pressure is one of the "colligative" properties of a solution, i.e. it is affected by the number of discrete "particles" (molecules and/or ions) in solution, irrespective of their size. It is related to the other colligative properties of a solution, namely freezing point depression, vapour pressure lowering and boiling point elevation, and may be measured indirectly by means of these.

Freezing point depression represents one of the longest established and most convenient methods of measuring the osmotic pressure of a solution. The first apparatus for determining freezing points with any

degree of sensitivity was that devised by Beckmann (1888). Since then, many technical advances have been made, and there are now many precision instruments available for measuring freezing point depression with a high degree of accuracy. Many of these instruments are calibrated so that readings may be obtained directly in units of osmotic pressure. The unit of osmotic pressure is the osmol, and the concentration of a solution expressed in osmoles per Kg. solvent is termed osmolality.

Osmolality = ϕn moles, where ϕ is the osmotic coefficient, and n is the number of particles formed upon dissociation of the solute in solution (Wolf, 1962). In most biological fluids, the osmolality is more appropriately measured in milliosmol units (1 osmol = 1000 milliosmoles).

All plant and animal cells contain solutions of salts, sugars, and macromolecules, enclosed in membranes which are essentially semi-permeable. Consequently, osmosis and osmotic pressure play an important role in the exchange of water and dissolved materials between cells and their extracellular environment. If cells are suspended in solutions of different concentrations it is possible to obtain a concentration at which neither shrinkage nor swelling of the cells will occur, i.e. where fluid neither passes into nor out of the cell. This solution has therefore the same osmotic pressure as the cell when separated from it by a semi-permeable membrane and is termed an isosmotic solution. The magnitude of osmotic forces is considerable, and when plasma, interstitial fluid and cell fluid are separated from pure water by a semi-permeable membrane, a force of the order of 6.7 atmospheres at 37° is required to prevent transfer of water

across the membrane (Pitts, 1963a). The magnitude of these forces is responsible for the rapidity with which water is distributed through capillary walls and cell membranes, thus preserving essentially equal osmolar concentrations in the extracellular, interstitial, and intracellular fluids.

Osmosis, osmotic pressure and the theory relating to them are physico-chemical concepts, but as a result of their application to living systems, these terms are widely used in biological sciences. It should be pointed out that there are certain differences between the physico-chemical and biological interpretation of these terms. In the strict physico-chemical definition, an isolated solution has no osmotic pressure as such. Only if the solution is separated from its solvent by a semi-permeable membrane will any osmotic pressure exist. A looser terminology has developed in biological fields, which permits the application of osmotic pressure to an isolated solution. A discussion of the biological viewpoint and the justification for it is given by Bayliss (1931). In biological work, osmotic pressure is taken as an expression of the potential pressure which would be required to prevent solvent flow into a solution via a semi-permeable membrane. This permits the application of osmotic pressure to isolated extracellular fluids and solutions.

Role of the Kidney in Homeostasis.

The maintenance of the normal composition and osmotic pressure of the extracellular fluid is essential to preserve the tonicity of living cells. This requires some regulatory mechanism to compensate for any

changes that occur in the composition and osmotic pressure of the extracellular fluids. The major organ which brings about this regulation is the kidney, which maintains the homeostasis of the body fluids by varying the output and the composition of the urine. The osmotic pressure of the extracellular fluids, and hence that of the cells they bathe, is maintained in the region of 290 mOsm. per Kg. water. The human kidney is capable of producing urine which can vary in osmolality from about 50 mOsm. per Kg. water under conditions of maximal diuresis to 1400 mOsm. per Kg. water under conditions of antidiuresis (Berliner, Levinsky, Davidson and Eden, 1958).

The volume of the urine produced by the kidney is determined largely by the needs of the body to conserve water. In response to the need for water conservation, the posterior pituitary gland secretes the antidiuretic hormone (Gilman and Goodman, 1937). An increase in plasma osmolality initiates release of this hormone by stimulation of osmo-receptors in the hypothalamus, causing nerve impulses to be relayed to the posterior pituitary which produce a secretion of antidiuretic hormone (Verney, 1947). The target organ for the action of this hormone is the kidney, and the site of action is the distal and collecting tubules of the kidney.

In the presence of antidiuretic hormone, the membranes of the distal and collecting tubules are permeable to water, and water reabsorption occurs at these sites, resulting in a small volume of concentrated urine being produced. In the absence of antidiuretic hormone, the membranes of the distal and collecting tubules are relatively impermeable to water, and almost no water reabsorption occurs, resulting in a large volume of dilute urine.

Urine Formation by the Kidney.

Theories relating to urine formation evolved mainly from developments in the study of renal histology and morphology. Attempts were made to relate the various structural units within the kidney to their possible functional role in urine formation. The functional unit of the kidney is the nephron consisting of the glomerulus, proximal tubule, descending and ascending limbs of the loop of Henle, the distal tubule and collecting tubule.

Ludwig (1844) developed one of the first theories of urine formation, in which he postulated that urine formation began with the separation of a protein free ultrafiltrate of blood plasma by the glomerulus. The filtrate was thought to be reduced in volume as a result of reabsorption via the renal tubules, hence resulting in concentration of the excretory products. Heidenhain (1874) proposed urine formation by a combination of both glomerular filtration and tubular secretion, the latter process occurring by transfer of material from the peritubular capillaries into the lumen of the tubules. Confirmation of these early views was possible when micro-puncture techniques were developed which permitted removal and analysis of fluid from various sites within the nephron.

Richards (1920) confirmed that the fluid emerging from the glomerulus was an ultrafiltrate of plasma with respect to osmotic pressure and various other criteria. He also proved that tubular reabsorption occurred. At the end of the proximal tubule, the volume of the glomerular filtrate was reduced by about 80%, while the osmotic pressure was still equal to that of the plasma. This proved that water reabsorption must have been accompanied

by solute reabsorption. Smith and his colleagues demonstrated that the main solutes actively reabsorbed in the proximal tubule were sodium and chloride, accompanied by an isosmotic amount of water (Smith, 1947; Wesson, Anslow and Smith, 1948).

Evidence of tubular secretion came initially from studies on the rate of excretion of the indicator dye phenol red, which was rapidly excreted in the urine after administration. Marshall and Vickers (1923) observed that about 60% of the dye was bound to plasma albumin, and hence could not be filtered through the glomerulus. The rate of excretion of the dye was too high to be explained by glomerular filtration, and they concluded that tubular secretory mechanisms were involved. The three discrete processes involved in the urine formation are, therefore, glomerular ultrafiltration, tubular reabsorption and tubular secretion.

The Renal Concentrating Mechanism.

The above mentioned processes did not account for the mechanism by which a concentrated urine was produced nor did they assign any specific functional role to the loop of Henle. This structure has been linked with the concentrating function of the kidney, since Peters (1909) noted a correlation between the maximal concentration of urine achieved by various mammals and the length of the thin segment of the loop of Henle. In 1927, Crane pointed out that only mammals and birds can form a concentrated urine, and only in these species did the thin segment of the loop of Henle occur. The function of the loop of Henle defied explanation for a considerable period, since fluid emerging from the loop was shown by micro-puncture studies to be hypotonic or isotonic, and this appeared to contradict the

idea that this structure was involved in the concentrating process.

In 1951, Wirz, Hargitay and Kuhn postulated a new theory which explained the function of the loop of Henle and provided a mechanism for the production of a concentrated urine. The basis of this theory depended on the unique spatial and anatomical arrangement of the various structures within the kidney. A simplified diagram of a nephron is shown (Fig. 1), illustrating the relationship of the various structures involved. A spatial arrangement in which part of the system bends back on itself and moves in the opposite direction allows the operation of a counter current multiplier system. The basic feature of such a system is that two streams of fluid moving in a counter direction, and in reasonable proximity to one another e.g. descending and ascending limbs of the loop of Henle, allows an exchange of material between the two streams of fluid.

The descending and ascending limbs of the loop of Henle and the arterial and venous limbs of the capillaries in the medulla would constitute such a counter current system. This system requires some initial active process to create a concentration difference between the two limbs. The active process involved is thought to be removal of sodium salts from the ascending limb, and their deposition in the interstitial fluid of the inner medulla. This increased concentration in the interstitial fluid would result in diffusion of water from the descending limb and hence the concentration in the descending limb would increase. The result of this continuous action would be zones of increasing hypertonicity in the interstitial fluid of the inner medulla, directed towards the renal papilla.

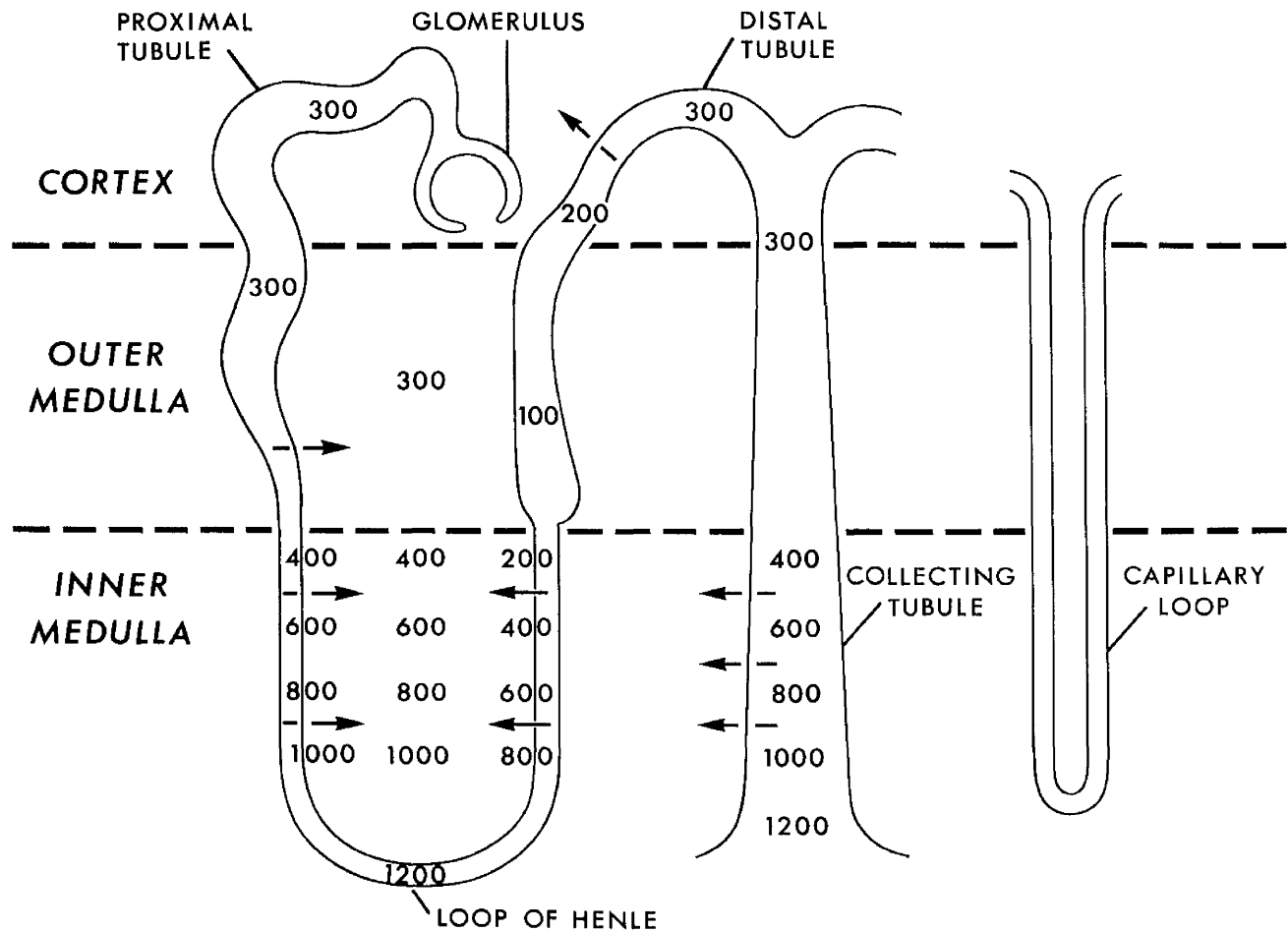


FIGURE 1. THE NEPHRON: ESTABLISHMENT OF AN OSMOTIC GRADIENT DURING THE PRODUCTION OF A HYPERTONIC URINE.

→ Active transport of salts.

- → Passive water diffusion.

Figures denote the osmotic concentration (mOsm. per Kg. water) at the various levels within the nephron.

Fluid emerging from the loop of Henle would pass through the distal tubule and then during its passage down the collecting duct, it would traverse this osmotic gradient produced by the loop of Henle. During antidiuresis, the action of the antidiuretic hormone would result in the collecting duct being permeable to water and so water would be lost during the passage along the osmotic gradient of the inner medulla, resulting in the production of a concentrated urine.

A review of this mechanism is given by Berliner, Levinsky, Davidson and Eden (1958). More recently, Sabour, MacDonald, Lambie and Robson (1964) as a result of electron microscope studies, proposed that antidiuretic hormone is responsible for the removal of water from the descending limb of the loop of Henle, and that a counter current system operates only in the concentrating process and not in the diluting process. During the concentrating process, the counter current flow of capillary blood through the inner medulla allows the osmotic gradient to occur within the inner medulla, without a significant increase in the concentration of the blood emerging from this region.

Urea Handling by the Kidney.

One of the major solutes which the kidney concentrates and excretes is urea. Urea has long been known to play a considerable role in the urine concentrating process (Gamble, McKahnn, Butler and Tuthill, 1934). For example there is considerable evidence that on a low protein diet, the concentration of non-urea solutes is lowered as well as urea concentration (Epstein, Kleeman, Pursel and Hendrikx, 1957; Jaenike, 1964).

Urea accumulates within the renal medulla during antidiuresis (Berliner et al., 1958) but very little accumulation of urea occurs during water diuresis (Ullrich and Jarausch, 1956). It is thought that the collecting duct is the source of this urea, and because of its diffusibility, urea as well as water diffuses out of the collecting duct during antidiuresis. In support of this, Jaenike (1961) showed that vasopressin (a purified preparation of pituitary extract with antidiuretic activity) increased the permeability of the collecting ducts to urea. Carlisky, Brodsky and Huang (1962) produced evidence of urea biosynthesis in the kidney by cortical and sub-cortical cells.

The accumulation of urea in the kidney during antidiuresis provides an explanation for the observations of Shannon (1936) on the effects of changing urine flow on urea excretion. The elevation of urea clearance above the steady state value when the urine flow rate is rising (Moller, McIntosh and Van Slyke, 1928 (a)), probably represents the removal of urea which has accumulated in the medulla during antidiuresis.

General Methods of Assessing Renal Function.

The earliest tests of renal function followed the recognition of nephritis by Bright in 1829. The initial studies were concerned with differentiating subjects with Bright's disease from normals. The first observation of a raised blood urea associated with this condition was made by Christison (1829) who found that in 3 out of 6 patients with Bright's disease, there was an increased serum urea concentration. In 1836, Bright reported a serum urea of 1500 mg. per 100 ml. in a uraemic patient.

The significance of abnormal urine findings was recognised, and in particular, the frequent occurrence of albuminuria associated with this condition. Following on from these initial studies, much of the investigation in the latter half of the 19th century was concerned with morphological and circulatory changes associated with nephritis.

At the beginning of the present century, interest was revived in the functional aspects of renal disease. Blood urea measurement was a relatively insensitive index of renal function. The determination of the urea excretion rate together with blood urea was introduced to give some quantitative indication of renal impairment. This initiated the use of the clearance formula $\frac{U \times V}{B}$, where V is the urine flow rate, U is the urine concentration and B the blood concentration of a given solute. This clearance value gave a measure of the number of ml. of blood cleared of solute per minute. This general formula could be applied to the clearance of any solute excreted in the urine. In relation to urea clearance, it was recognised that other factors being constant, the blood urea concentration was proportional to the rate of protein catabolism, but the effect of urine volume changes on urinary urea concentration was not initially recognised. The existence of an upper limit of urine flow rate, above which the rate of urinary urea excretion was directly proportional to the blood urea concentration was first shown by Austin, Stillman and Van Slyke (1921). The upper limit in normal adults was at urine flow rates of 2 ml. per min. At flow rates greater than this, the clearance of urea was termed maximum clearance. At flow rates less than this upper limit, the rate of urea excretion was proportional to the square root of the urine flow rate and the clearance of

urea was termed standard clearance. These findings were confirmed by Moller, McIntosh and Van Slyke (1928 (a)) in additional normal subjects. The average normal maximum clearance was 75 ml. per min., and the average normal standard clearance was 54 ml. per min.

When both the standard and maximum urea clearances were expressed as a percentage of the average normal, a measure of renal function was obtained. This urea clearance test was applied to nephritic patients by Moller, McIntosh, and Van Slyke (1928 (b)) who found that reduced renal efficiency was present in many patients. A comparison of standard and maximum clearances in the same subjects indicated that either clearance was equally sensitive in the detection of renal impairment. The results also confirmed the findings of MacKay and MacKay (1927) that a loss of up to 60% of renal function could occur with a blood urea concentration still within the normal limits.

The excretion of urea by the kidney depends both on glomerular filtration and tubular reabsorption and consequently urea clearance depends on the integrity of both these functions. The urea clearance is still widely used in the clinical assessment of renal function. One of its main advantages is that it can be easily carried out as a routine investigation, and urea concentrations can be determined simply and accurately in any routine biochemical laboratory.

The introduction of clearance methods enabled a quantitative assessment of renal function to be made in both normals and renal disease patients. It also made possible follow-up studies in renal disease cases and revealed the progression of the disease. One possibility which was soon explored was that clearance studies using substances handled differently by the kidney would

make it possible to differentiate lesions associated with different functional areas of the kidney. Urea clearance depended both on glomerular filtration and tubular reabsorption. If, however, a substance could be found which was neither reabsorbed nor secreted by the tubules, then the clearance of this substance would depend only on glomerular filtration and would give a measure of glomerular filtration rate. In 1926, Rehberg introduced the clearance of endogenous creatinine as a measure of glomerular filtration rate, since creatinine represented the physiological constituent of plasma which was most concentrated in the urine and was not reabsorbed to any extent by the kidney tubules. This test is still used as a measurement of glomerular filtration rate, but it has certain disadvantages. The method of estimation of serum creatinine is inadequate, since the Jaffé chromogen which is estimated is not specific for creatinine and therefore serum values based on this method may be erroneously high. In addition, it has been shown that in man, a small amount of creatinine is secreted by the tubules, and in patients with proteinuria, the tubular secretion of creatinine appears to be increased (Berlyne, Varley, Nelwarangkur, and Hoerni, 1964).

In 1935, Shannon and Smith proposed the use of the fructose polysaccharide, inulin, which had all the properties required of a substance to be used as a measure of glomerular filtration rate. The practical application of the inulin clearance test is severely limited, however, since inulin must be administered by intravenous infusion at a constant rate. Urine collections must be accurate and complete, and ideally, the patient should be catheterised. On theoretical grounds, inulin clearance

represents an ideal method of measuring glomerular filtration rate, but the elaborate procedures involved make it unsuitable for ordinary clinical investigation.

It has been shown that there is not a constant relationship between inulin and creatinine clearance in man (Berlyne et al., 1964). The value of the creatinine clearance is very often higher than that of inulin, due to tubular secretion of creatinine. More recently, Nelp (1964) proposed the use of a radioactive form of Vitamin B₁₂ in the measurement of glomerular filtration rate in man. There was a good correlation between the clearance of radioactive Vitamin B₁₂ and inulin clearance in both normal subjects and patients with impaired renal function.

Tests based on Urinary Concentrating Ability.

Clearance tests all depend on the excretion of one particular substance and there would be some advantage in a test which measured the ability of the kidneys to handle solutes in general. For this reason, much interest centred round the measurement of urine specific gravity since this gave a measure of the total concentration of solutes present in the urine. Following periods of fluid deprivation, normal subjects excrete a concentrated urine with a high specific gravity, and use has been made of this in various tests of renal function based on the measurement of urine specific gravity. One of the first tests of renal concentrating ability based on specific gravity was that introduced by Addis and Shervy in 1922. The Fishberg concentration test (1954) is typical of the various specific gravity tests which were used for routine clinical application. Subjects were deprived of fluids for a 14 - 15 hour period overnight, and then urine was collected for specific

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gravity measurement over two one-hour periods. Results were considered abnormally low if subjects could not attain a specific gravity of 1.024 under these conditions.

The measurement of specific gravity by a clinical hydrometer represents a relatively insensitive method, since it is incapable of distinguishing minute differences in different samples. All measurements must be made at a constant temperature since specific gravity varies with temperature. Specific gravity is not a colligative property and is therefore affected by the size of particles present in solution. In particular the presence of protein or sugar in urine gives a falsely high estimate of concentrating ability. This limits its application as both albuminuria and glycosuria are frequently found in renal disease patients.

In the measurement of urinary concentrating ability, osmolality is more meaningful than specific gravity. The kidney responds to changes in the osmolality of the body fluids but is not affected by changes in specific gravity, consequently the measurement of osmolality has a more physiological basis as an indication of concentrating ability. In addition, osmolality is one of the colligative properties and therefore is affected by the total number of particles present in solution, irrespective of their size. With the development of techniques for measuring accurately the osmolality of small quantities of fluid, urinary osmolality has superceded specific gravity as an indication of urine concentration. *

In several studies, comparisons have been made between specific gravity and osmolality (Rapoport, Brodsky, West and Mackler, 1949; Hopper, Bolomey and Wennesland, 1954). In general, a poor correlation has been found between

the two, due to the inadequacy of the methods of measuring specific gravity and the fact that many of the measurements were carried out on renal disease patients with proteinuria and glycosuria. Frank, Dreifus, Rarick and Bellet (1957) found that in subjects with neither protein nor sugar in the urine, a reasonable correlation was obtained between the two, provided specific gravity was measured carefully using an ordinary clinical hydrometer.

Normal subjects undergoing a water diuresis will produce a dilute urine with low osmolality; only at low urine flow rates will a maximally concentrated urine be produced. Consequently, some form of fluid restriction is required in any test of renal concentrating ability. Most of the tests based on osmolality were carried out under similar conditions to the specific gravity tests in which subjects were deprived of fluids for a considerable period prior to the test.

Dehydration provides a very variable stimulus for renal concentration of the urine and its effectiveness depends on many factors such as environmental temperature, the activity of the subject, the rate of insensible water loss via the skin. In the literature, opinions differ considerably as to the optimal length of dehydration period required to produce a maximally concentrated urine. A period of 48 hours fluid deprivation was used by Miles, Paton and de Wardener (1954), and by Jones and de Wardener (1956) in their studies on patients with normal renal function and normal active subjects. Zak, Brun and Smith (1954) recommended a period of 12 - 14 hours dehydration, and Baldwin, Berman, Heinemann and Smith (1955) used a 15 - 18 hour dehydration period. The majority of tests favoured 12 - 14 hours, since this could be conveniently carried out by withholding fluids from about 8 p.m. overnight,

and collecting two hourly samples from 8 a.m.

In normal subjects, fluid deprivation for long periods is unpleasant, and in patients with renal impairment who are unable to conserve water normally, such periods of dehydration lead to considerable discomfort. As a result, the use of long acting preparations of vasopressin has been advocated to stimulate renal conservation of water, while allowing subjects free access to fluids. However, this process is not completely equivalent to dehydration, and it has been found that in normal subjects, the maximal concentration is greater after fluid deprivation, than after vasopressin alone (Jones and de Wardener, 1956; Epstein, Kleeman and Hendriks, 1957).

Following either water deprivation or vasopressin, the index which has been most commonly used as measure of renal concentrating ability is the ratio of urine to plasma osmolality or simply the urine osmolality. Frank et al., (1957) compared results obtained using this urine:plasma osmolality ratio, with other tests such as blood urea nitrogen and the Fishberg concentration test, in normal subjects and renal disease patients. Abnormal concentrating ability was detected in many patients who had normal results as judged by other tests. The plasma osmolality was found to be relatively constant, and since it did not significantly alter the results, they favoured the use of urine osmolality alone as an index of renal concentrating ability. In normal subjects, the minimal urine osmolality recorded was 800 mOsm. per Kg. water and the extent to which the osmolality of renal disease patients was less than this, was roughly proportional to the severity of the disease. They suggested that this test could be used as

a graded quantitative method of evaluating renal function changes. In the early stages of renal impairment, concentrating ability appeared to be a more sensitive index of renal function than many of the other recognised tests.

In a similar study, De Leon, Dreifus and Bellet (1960) investigated concentrating ability in a group of 36 patients with hypertension. In 32 of the patients, urine osmolality was less than 800 mOsm. per Kg. water, indicating impairment of renal concentrating ability. They were unable to correlate the degree of impairment, with the severity of the hypertension. Jacobson, Levy, Kaufman, Gallinek and Donnelly (1962) studied concentrating ability in normals, and in patients suffering from various forms of acute and chronic renal disease. In normal subjects, the minimal urine:plasma osmolality ratio was 3.0, and 47 out of 58 patients were unable to attain this. No attempt was made to relate the urine:plasma ratio with the degree of impairment, and the test was used mainly to differentiate normals from abnormals.

A different assessment of renal function based on osmolality measurement was used by Baldwin et al. (1955). The urinary concentrating mechanism was measured using the term $T_m^0 H_2O$ which represents the amount of solute free water required to restore the urine to an isosmotic state. The osmolar clearance C_{osm} was calculated i.e. the volume of isotonic urine which would have resulted in the excretion of the same amount of solute. The difference between C_{osm} and the observed volume V was designated $T_m^0 H_2O$ and was used as a measure of concentrating ability. Patients were given vasopressin to stimulate maximal tubular reabsorption of water, and inulin clearances were

determined simultaneously. In patients with glomerulonephritis, there was evidence of a concentrating mechanism, and in the early stages, the damage appeared to be predominantly glomerular. In acute renal failure, a marked reduction in concentrating ability was found, and this was out of proportion to the reduction in filtration rate.

In general, tests based on osmolality showed that there was a defect in renal concentrating ability in many different forms of renal disease. This defect could be detected using the urine osmolality, urine:plasma osmolality ratio and in some cases using $T_m^C H_2O$. In many cases, abnormal concentrating ability was detected when other tests gave normal results.

Application of tests based on concentrating ability to conditions other than renal disease.

Apart from their use in the detection of renal impairment, tests of renal concentrating ability have been used in the diagnosis of various other clinical conditions. The ultimate concentration of the urine depends on the renal tubular reabsorption of water under the influence of the antidiuretic hormone. Consequently, conditions in which secretion of this hormone is impaired are associated with abnormal renal concentrating ability. In particular, in diabetes insipidus there is no secretion of antidiuretic hormone by the posterior pituitary, and large volumes of dilute urine are produced in this condition. Dashe, Cramm, Crist, Habener and Solomon (1963) described a water deprivation test by which normal subjects could be distinguished from diabetes insipidus patients and patients with mild pituitary damage. The characteristic finding in diabetes insipidus patients was that following a $6\frac{1}{2}$ -hour period of water deprivation, all

patients had an abnormally high serum osmolality and an abnormally low urine:plasma osmolality ratio. In normal subjects and in patients with only mild pituitary damage serum osmolality was unchanged. The average urine:plasma osmolality ratio was 3.8 in normals, and was decreased in patients with mild pituitary damage.

There is also evidence of an apparent renal defect as judged by renal concentrating ability in subjects with both sickle cell anaemia, and sickle cell trait (Zarafonitis, McMaster, Moltman and Steiger, 1956). All subjects gave normal results for other renal function tests, but the maximal urine osmolality following 18 hours fluid deprivation was considerably sub-normal, and averaged 421 mOsm. per Kg. water in sickle cell anaemia, and 604 mOsm. per Kg. water in sickle cell trait. There is little evidence at present to indicate the cause of the concentrating defect in this condition.

Factors Affecting Urine Concentration.

Innumerable factors are known to affect the concentration of urine, and hence urine osmolality in normal subjects. If urine osmolality is to be taken as a valid index of renal concentrating ability, considerable attention must be given to any factors which are likely to interfere with normal concentration.

(i) Fluid intake:

Fluid deprivation is one of the most effective methods of producing a high osmolality, concentrated urine. This occurs as a result of stimulation of secretion of the antidiuretic hormone. A similar effect to dehydration can be produced using vasopressin. However, it has been

shown that urine concentration was greater after dehydration, than after administration of vasopressin (Jones and de Wardener, 1956).

The effects of high fluid intakes on urine concentration are considerable. In addition to reducing urine osmolality during the excretion of a water load, there is evidence that following a period of high fluid intake renal concentrating ability is impaired (Epstein, Kleeman and Hendrikx, 1957 and de Wardener and Herzheimer, 1957). The effect of high fluid intake for prolonged periods was studied by Habener, Dashe, and Solomon (1964) in normal subjects over a 6-week period of increased fluid intakes. At the end of this period, serum osmolality was not significantly different from the control periods, indicating that the water regulating mechanism was unaffected. When subjects were deprived of fluids, however, and renal concentrating ability measured, it was found to be considerably less than in the control periods.

(ii) Dietary intake:

Variation in dietary intake affects the renal concentrating process. Meroney, Rubini and Blythe (1958) compared the effects of four different antecedent diets on the renal concentrating process (a) normal, (b) low protein and low salt, (c) high protein and low salt, (d) high salt and low protein. Using urine osmolality after overnight dehydration as the index of renal function, they found that the highest value was associated with diet (c) and the lowest with diet (d). Epstein, Kleeman, Pursel and Hendrikx (1957) studied the administration of both protein and urea to normal dehydrated and overhydrated subjects and found that chronic administration of either augmented the renal concentrating process.

As would be expected, low protein diets are associated with low urea clearances, similarly high protein diets with increased urea clearances. The mechanism involved in this process is uncertain. Nielson and Bang (1948), studying the influence of diet on normal subjects, claimed that the fall in urea clearance on low protein diets was associated with tubular factors and that glomerular filtration rate was unchanged. Pullman, Alving, Dern and Lansdowne (1954) studying renal function in normal subjects on different diets found that low protein diet decreased, and high protein diet raised, the glomerular filtration rate. More recently, Kleeman, Radford and Torelli (1965) in a study of simultaneous urea and inulin clearances in rats found that the increase in urea clearance was greater than that in glomerular filtration. This suggested that some factor other than filtration is responsible for the elevated urea clearance on a high protein diet.

(iii) Emotional Factors:

The innervation of the kidney by the sympathetic nervous system has an important role in emergency situations. Severe stress stimulates vasoconstriction via this nervous supply to the kidney, reducing renal blood flow and allowing greater temporary perfusion of more vital organs such as the heart and the brain. The release of antidiuretic hormone is also affected by certain emotional stimuli such as fear and pain, resulting in oliguria. Even relatively mild forms of emotional stress have been shown to alter renal blood flow in normal subjects. Pfeiffer and Wolff (1950) found that discussion of topics liable to produce emotional stress produced a reduction in renal blood flow. In the same study, changes in

hypertensive subjects were found to be more marked than in normals.

In general, emotional factors appear to have a more profound effect on renal excretion in subjects with psychological disturbances. Schottstaedt, Grace and Wolff (1956) found that situations of depression, and emotional stress in a group of psychologically disturbed patients were associated with decreased rates of excretion of water, sodium and potassium. In a study of severely depressed subjects, Coppen and Shaw (1963) demonstrated a significant increase in intracellular sodium during depression. Barnes and Schottstaedt (1960) observed wide variations in fluid and electrolyte excretion in patients with congestive cardiac failure, associated with attitudes and emotions.

In contrast, in normal subjects at rest both physically and mentally, maintained in a relatively constant environment, the renal nerves transmit almost no impulses, and consequently, vasoconstrictor activity is minimal (Smith, 1951). Under such conditions, concentrating ability should be unaffected.

(iv) Postural changes:

Change of posture from the supine to the standing position results in a slight antidiuresis in the standing position (Pearce and Newman, 1954). Strauss, Davis, Rosenbaum and Rossmesl (1951) showed that expansion of the extracellular fluid produced by infusion of isotonic saline would produce a diuresis in recumbent subjects with no significant effect when given to subjects in the sitting position. The diuresis associated with recumbency was similar to a water diuresis, in that excretion of water occurred in excess of solute.

(v) Exercise:

(v) Exercise:

in normal hydropenic men, periods of heavy exercise were associated with reduced concentrating ability due to decreased solute excretion, particularly sodium excretion (Raisz and Scheer, 1959). The urine:plasma osmolality ratio decreased markedly in the first hour after exercise, and the urine volume was lower during this period than in the control period. This reduction in concentrating ability could not be prevented by administration of vasopressin. If the normal renal concentrating process depends on the establishment of a concentration gradient of sodium salts in the medulla, any reduction in the delivery of sodium salts to this region would limit the concentrating ability.

(vi) Temperature:

A reduction in environmental temperature is known to produce a diuresis and hence to reduce renal concentrating ability. The mechanism of this "cold diuresis" was studied by Bader, Eliot and Bass (1951) on nude, recumbent males exposed to a temperature of 16°. The diuresis resembled a water diuresis in that urine specific gravity fell; there was no significant change in glomerular filtration rate, but the renal tubular reabsorption of water was diminished. Small doses of vasopressin produced an effective inhibition of the diuresis. The increase in urine output was greater than could be accounted for by the excretion of water normally lost via the skin.

(vii) Alcohol:

The diuretic action of alcohol in man is well known. The mechanism of this diuresis has been studied by various workers (Eggleton, 1946; Strauss, Rosenbaum and Nelson, 1950) and most of the evidence suggests

that the diuresis is due to the depressant action of alcohol on the hypothalamic centre responsible for the output of antidiuretic hormone. Normally, a reduction in the osmolality of body fluids inhibits antidiuretic hormone, and an increase stimulates its release. A study by Roberts (1963) in normals after alcohol ingestion and in chronic alcoholics showed that the water diuresis continued after alcohol ingestion despite an increase in serum osmolality. In chronic alcoholics, the increase in serum osmolality was more marked. The increase was partly accounted for by the increased concentration of blood alcohol. One curious feature was that none of the subjects experienced thirst while the concentration of blood alcohol was raised, suggesting that alcohol also inhibits the thirst centre.

(viii) Smoking:

The antidiuretic action of nicotine in man was demonstrated by Burn, Truelove and Burn (1945). Walker (1949) showed that the effect of two cigarettes deeply inhaled was comparable to 1 - 1.5 mg. intravenous nicotine in producing an antidiuresis. Nicotine given in similar doses to patients with diabetes insipidus produced no antidiuresis (Cates and Garrod, 1951), confirming that the action of nicotine most probably involved stimulation of antidiuretic hormone secretion.

(ix) Age:

Renal function alters with age. Infants are unable to attain as dilute urine as adults when given a water load, and similarly cannot reach the same maximal concentration under dehydration. After the age of 40 years, there is a decline in the weight of the human kidney, and this is often associated with a slight reduction in certain functional activities (McCance, 1962).

(x) Changes in urinary composition in the extra-renal collecting system:

Changes in urine during ureteral passage were studied by Garby, Risholm, Thoren and Ulfendahl (1957). They found that the changes during ureteral passage varied considerably in different individuals, but there was a general tendency to a lower urinary concentration in the lower end of the ureter. The changes in concentration seemed to be partly the result of diffusion of different substances across the ureteral mucosa. Leaf (1960) in a review on this topic, pointed out that changes also occur during accumulation of urine in the bladder, and fluid collected from the bladder could have an osmolality 10% less than fluid entering the ureter. This effect was more pronounced, the longer the accumulation period in the bladder, and the lower the urine flow rate. Leaf pointed out that these changes could give rise to slight inaccuracies of renal function estimation, based on examination of bladder urine. However, where collection periods are standardised and relatively short, and urine flow rates similar, this error would presumably be reasonably constant in a group of subjects.

(xi) Diurnal variation:

The pattern of excretion of water and of the major urinary solutes varies throughout the day. During the evening, and throughout the night, the urine flow rate diminishes, while throughout the day it increases. As a result of this variation, the day-time urine output amounts to about twice the volume of night urine. A similar decrease during the night and increase during the day is found in the excretion patterns of sodium and chloride (de Wardener, 1961). Martel, Sharp, Slorach and Vipond (1962), showed that

the increase in excretion of water, sodium and chloride throughout the day was not due to the increased food and fluid intake, exercise, or change of posture. They demonstrated that this pattern was maintained in patients recumbent in bed, who were given food and water regularly throughout the 24 hours. The pattern of urea excretion is inversely related to the urine flow pattern, with maximal values during the night, and a decrease during the day (Menzel, 1962).

One consequence of this diurnal rhythm is that in the early morning, urine flow rate is lower and urine concentration is higher than later in the day. As a result, the period from early morning to mid-day is more suitable for urine collections, when low urine flow rates and concentrated urine specimens are required.

Changes in the Diseased Kidney.

With the onset of renal disease, changes occur in both the structure and function of the kidney. Normally, primary changes in renal disease are structural, and only after the disease process is established are functional changes detectable. This is due to the considerable reserve capacity of the kidney. There are about one million nephron units in the kidney, and not all of these are required to cope with the normal renal load of solutes and fluids. In the initial stages of the disease, specific functional sites may be affected e.g. the glomerulus or the renal tubules. As the disease progresses, entire nephron units are involved, and the functional mass of the kidney as a whole becomes reduced. Irrespective of the initial disease process, patients in the terminal stages of chronic renal disease present a similar pattern of gross renal insufficiency.

Outline of the Present Investigation.

In the literature, there is considerable difference of opinion as to the optimal conditions under which urine osmolality may be used as an index of renal concentrating ability. The first part of the present investigation was concerned with the study of urine osmolality in normal subjects at different urine flow rates and under different states of hydration. The ultimate aim was the application of the findings to patients with chronic renal disease. It was hoped to define more precisely the limits within which urine osmolality could be used as an index of renal function, and to establish the minimal requirements with respect to the state of hydration of subjects.

The second part was concerned with the application of these findings to patients with chronic renal disease. Renal function was assessed in various groups of patients using a test based on urine osmolality which allowed a quantitative assessment of renal concentrating ability. The results obtained were compared with one of the generally accepted tests of renal function, i.e. the Van Slyke urea clearance test, since it was considered to give a general indication of renal function, and to be one of the most reliable and suitable tests for application to patients. The relationship between results from both tests was compared at different stages of renal disease to assess the reliability of the test based on concentration ability. The possible application of the concentrating ability test to other clinical conditions was considered.

MATERIALS AND METHODS.

OSMOLALITY AND FREEZING POINT DEPRESSION.

Freezing point depression differs from most of the other factors used as a measure of solute concentration such as specific gravity or refractive index, in that it varies with the total number of "particles" (molecules and/or ions) present in solution, irrespective of their size. This measure of concentration based on the total number of particles is defined as "osmolality" (Gamble et al., 1934; Wolf, 1962).

The osmolality of a solution differs from its molality only if the solute is partially ionised in solution. Osmolality is expressed in units known as osmols. One osmol corresponds to the concentration of any solute which will depress the freezing point of water by 1.859° . For a non-ionic solute, one osmol is equivalent to one mole. For a solute which is partially ionised in solution, one osmol will be less than one mole.

1 osmol = ϕn moles where ϕ = osmotic coefficient

n = number of ions into which the
molecule theoretically could dissociate.

This measurement of freezing point depression in terms of osmolality has been applied to biological fluids to obtain an estimate of the total osmotic pressure present in the fluids. In view of the concentration ranges encountered in most biological fluids the "milliosmol" unit is most frequently used, where 1 milliosmol = 0.001 osmol.

The measurement of osmolality by the method of freezing point depression.

In the present study, all measurements of osmolality were carried out using the Fiske Osmometer Model G.* This instrument measures the freezing

* Advanced Instruments, Inc., Newton Highlands, Mass., U.S.A.

point of a solution by means of a highly sensitive thermistor, the resistance of which is altered by minute temperature changes. The instrument is calibrated so that the osmolality of any solution can be read off directly. This is possible since increase in freezing point depression is linear with increase in osmolality. A diagram showing the major operational units of the osmometer is given in Figure 2.

A sample of fluid is placed in a 100 mm. test-tube, and the tube is then mounted in the bracket so that the thermistor at the tip of the temperature probe is centred in the sample. The alteration in the electrical resistance of the thermistor produced by a change in the temperature of the sample is recorded on a null-balance galvanometer. A stainless steel rod adjacent to the probe vibrates at low amplitude and serves as the stirring mechanism during the cooling procedure. The coolant in the bath is 50% (v/v) ethylene glycol, thermostatically controlled at a temperature of $-6^{\circ} \pm 2^{\circ}$. The sample tube is suspended just above the liquid in the bath, and a pumping mechanism pumps coolant against the outside of the tube during the cooling procedure.

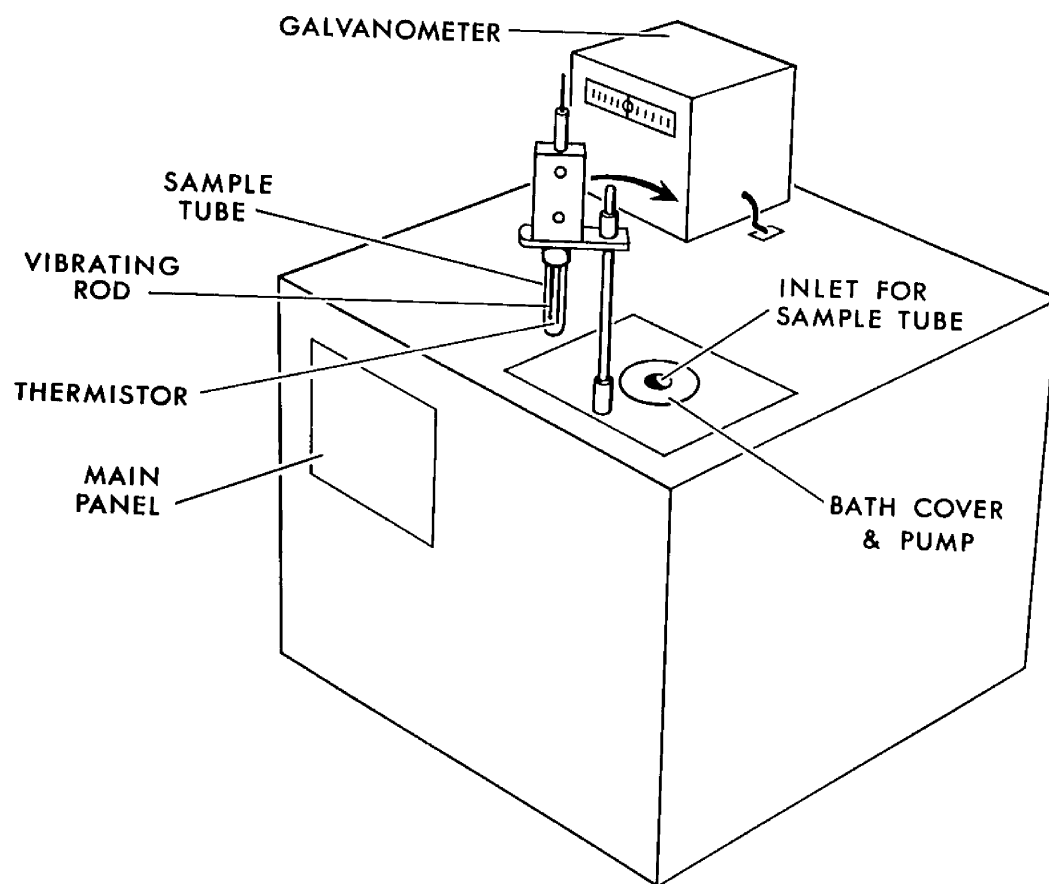


FIGURE 2.

FISKE OSMOMETER:--

MAJOR OPERATIONAL PARTS.

Method:-

A 2 ml. sample of the fluid is introduced into the test-tube and mounted as described. The bracket is then lowered so that the sample tube is suspended above the coolant level in the bath. The sequence of cooling, freezing and the temperature changes accompanying the various procedures are shown in Figure 3, and consist of:-

- (i) An initial fast-cooling period at a rate of 0.5-2.0 mm. per sec. on the galvanometer.
- (ii) A period of supercooling at a slower rate to allow the sample to reach a uniform temperature throughout.
- (iii) At a pre-selected point, 20 mm. to the left of the zero on the galvanometer scale, freezing is induced by sending a sharp electrical impulse through the coil which produces a violent one-second vibration in the stirring rod. This creates a number of nuclei throughout the specimen, at which crystallisation can take place.
- (iv) On freezing, the sample releases its latent heat of fusion. This change in temperature alters the resistance of the thermistor, which is recorded on the galvanometer. Since the thermistor is centrally located in the sample, it is insulated from the outside by the surrounding specimen, and therefore its temperature will remain relatively constant. All measurements are made when the sample has reached its plateau temperature, i.e. 30 - 45 seconds after freezing has been induced.

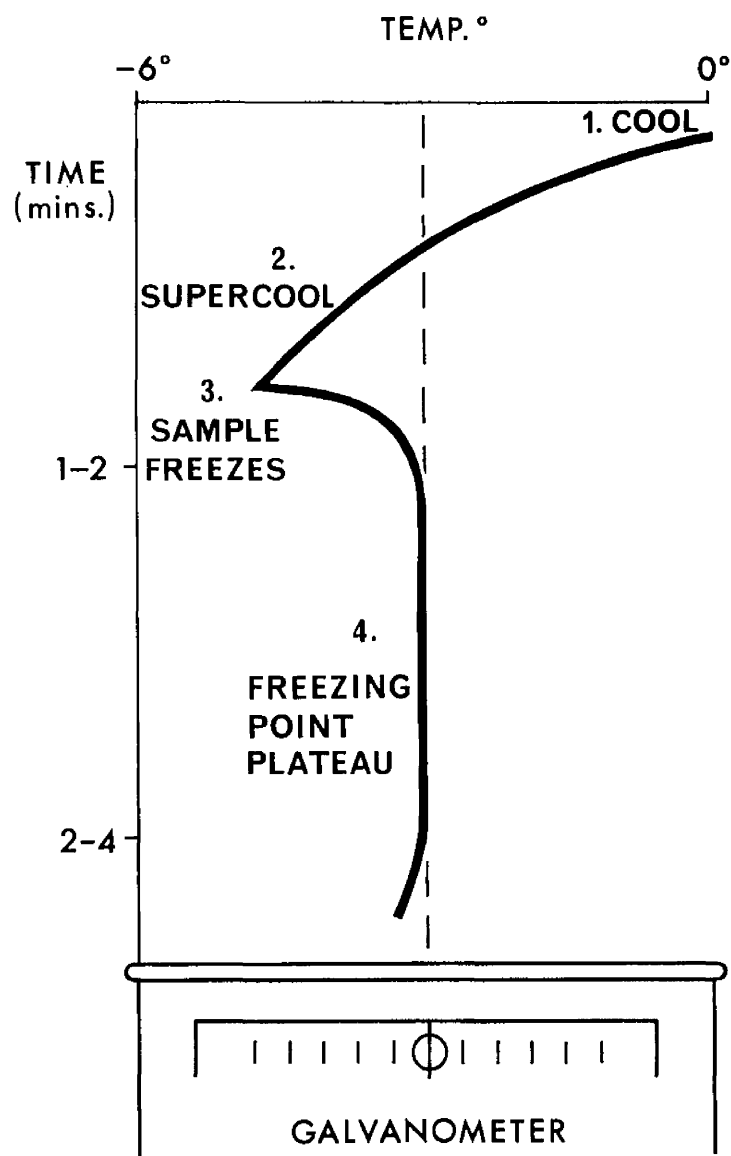


FIGURE 3.

A FREEZING CURVE USING THE FISKE OSMOMETER.

For pure water, the galvanometer light returns to the zero point of the scale on freezing; for a solution, the galvanometer is brought to zero by means of a dial which is calibrated in milliosmols and so gives a direct estimate of the osmolality.

Accuracy:- The accuracy of the instrument on repeat analyses is such that it will give an estimate of osmolality to within ± 1 milliosmol.

Calibration and Standards. In order to calibrate the machine for direct reading in a particular range, the use of two standards within the range is recommended. In the present work, where the range 0 - 1000 mOsm. per Kg. water was used, the machine was calibrated using two standards of osmolality 100 and 500 mOsm. per Kg. water.

The standards were aqueous solutions of sodium chloride. Analar grade sodium chloride* was used, recrystallised once from water, dried initially in an oven at 120° and finally in a desiccator over phosphorus pentoxide. In all calculations, allowance had to be made for the activity coefficient of sodium chloride at the various concentrations.

The required concentration of sodium chloride was calculated using the formula:- $C = 0.1086 \times \frac{\text{Osm.}}{k_o}$

where C = g. NaCl per Kg. water. Osm. = desired osmolality of standard
(mOsm. per Kg. water)

k_o = molal freezing point depression for sodium chloride at that concentration ($^{\circ}$ per mole per Kg. water)

0.1086 = Constant derived from $\frac{\text{M. Wt. NaCl} \times \text{Cryoscopic Constant for water}}{1000}$

Data for the two standards used is shown in Table I.

* British Drug Houses Limited.

TABLE I.

DATA FOR OSMOMETER STANDARDS IN THE RANGE

0 - 1000 mOsm. per Kg. water.

<u>Osmolality of standard</u> (mOsm. per Kg. water)	<u>Sodium chloride</u> (g. per Kg. water)	<u>Sodium chloride</u> (g. per l. water at 25°)	<u>Freezing</u> <u>point</u>
100	3.089	3.084	-0.186°
500	15.930	15.901	-0.93°

Specimens with osmolality greater than 1000 mOsm. per Kg. water.

When calibrated with the two standards of 100 and 500 mOsm. per Kg. water, the instrument is adjusted to direct reading only on the lowest range, 0 - 1000 mOsm. per Kg. water. Direct reading on higher ranges requires complete recalibration of the instrument using appropriately higher standards. The bulk of the specimens encountered were within the range 0 - 1000 mOsm. per Kg. water, and hence the instrument was calibrated for use in that range. There were however a few urine specimens with osmolality greater than 1000 mOsm. per Kg. water, and to permit the use of the same scale for these specimens, a dilution factor was calculated using a series of urine specimens and measuring the osmolality before and after 1 in 2 dilutions with distilled water. The true osmolality was related to the osmolality of the diluted specimen by a dilution factor F where

$$F = \frac{\text{True osmolality}}{\text{Osmolality of 1 in 2 dilution}}$$

For a series of 20 determinations, the mean value of F was 1.958 (s.d. \pm 0.0055)

This factor was applicable only to urine specimens with reasonably normal constituents. Any gross deviation from normal in the composition of the urine specimen e.g. chloride free urine, would obviously affect this dilution factor.

CONDUCTIVITY MEASUREMENT. The purpose of measuring conductivity was to obtain an overall estimate of urine electrolyte concentration in order to assess the contribution of electrolyte to urine osmolality in different specimens. The conductivity of undiluted urine specimens was measured

using a conductivity cell with platinum electrodes attached to a Wheatstone Bridge circuit. Measurements were made at 25⁰, and the cell constant (k) was derived using a potassium chloride solution (0.01 g. equiv. per litre). The specific conductivity for this concentration of potassium chloride at 25⁰ was 0.00141 ohms⁻¹cm⁻¹(K), and the observed conductivity was 0.00468 ohms⁻¹.

$$\begin{aligned}\text{The cell constant (k)} &= R \text{ (ohms)} \times K \text{ (ohms}^{-1}\text{cm}^{-1}\text{)} \\ &= \frac{0.00141}{0.00468} = \underline{0.301 \text{ cm.}^{-1}}\end{aligned}$$

This value was used in all subsequent measurements to convert observed conductivity readings to specific conductivity. The cell constant was checked from time to time, to ensure that no alteration in the characteristics of the electrodes had taken place.

An electrolyte standard was prepared, containing quantities of all the major urinary electrolytes based on their mean daily excretion (Varley, 1963, Blood and other Body Fluids, 1961). The composition of this standard is shown in Table II. In view of the wide normal range for excretion of many of the electrolytes, this standard would of necessity only approximate to any given urine specimen.

Since only ionic solutes were present, the osmolality of the standard was entirely contributed by electrolyte. The conductivity of the standard was measured, and various dilutions were made. A calibration curve relating the specific conductivity to the osmolality of the standard and dilutions is shown in Fig. 2 (cf. Beljan and Berry, 1961). Urea was then added to the standard in physiological amounts, and conductivity was measured to confirm

that the presence of urea had no depressant effect on electrolyte conductivity. It was therefore valid to use the conductivity of a urine specimen containing urea as an estimate of the total electrolyte content. From Fig. 4, it was possible to assess the contribution of urinary electrolyte to the total osmolality.

The estimation of blood and urinary constituents were performed by the following methods.

(a) Urea. Urea was determined on either whole blood or serum.

Urine specimens were diluted appropriately with distilled water. Using the Technicon* Auto-analyser, urea was estimated by a method based on the diacetyl monoxime reaction.

(b) Chloride. Urine chloride was measured by titration against mercuric nitrate, using diphenyl carbazone as indicator (Schales and Schaless, 1941). The urine was first acidified with a few drops of nitric acid.

* Technicon Instruments Co. Ltd., Surrey, Great Britain.

TABLE II.

COMPOSITION OF ELECTROLYTE STANDARD.

<u>NaCl</u>	-	7.84 g./l.
<u>KCl</u>	-	4.20 g./l.
<u>NaHCO₃</u>	-	0.16 g./l.
<u>NaH₂PO₄</u>	-	2.30 g./l.
<u>Na₂SO₄</u>	-	0.93 g./l.
<u>CaCl₂·6H₂O</u>	-	0.93 g./l.
<u>MgCl₂·6H₂O</u>	-	0.68 g./l.
<u>Osmolality of standard</u>	-	539 mOsm./Kg. water
<u>Specific conductivity</u>	-	0.0263 ohms ⁻¹ cm. ⁻¹

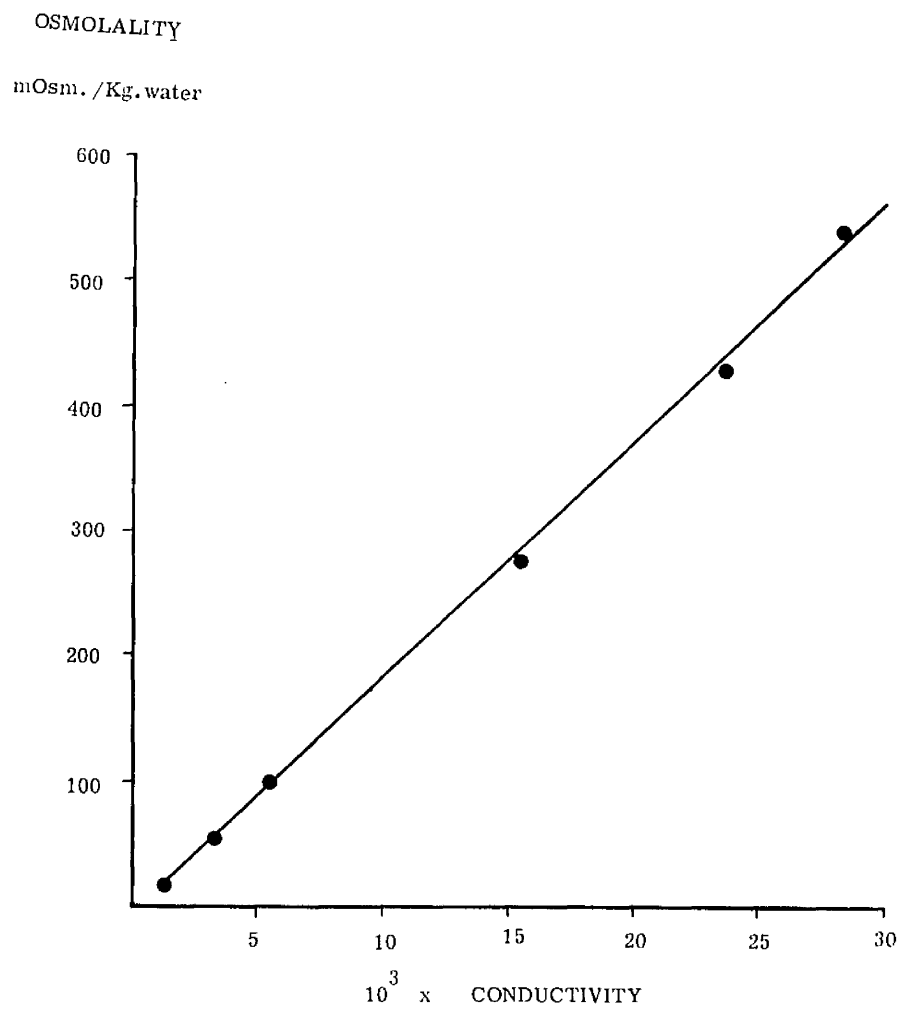


FIGURE 4. CALIBRATION GRAPH FOR ELECTROLYTE STANDARD.

EXPERIMENTAL CONDITIONS.

Most of the experiments depended on the collection of urine specimens at accurately timed intervals, and the measurement of urine volume, osmolality and various urinary constituents. In view of the many extraneous factors which affect particularly urine volume and concentration, a careful control of conditions and environmental factors was maintained during all tests. Some of the major factors likely to affect urine volume and concentration will now be considered, and the conditions of the tests and subjects will be outlined.

State of hydration of subjects. The individual response to periods of fluid withdrawal, and to administration of fluids is very variable. As a result, it is difficult to define with any precision conditions which would produce a similar state of hydration in a number of individuals. The following terms have been used to describe the state of hydration of subjects:-

- (i) Dehydration. Fluids were withheld for a 14-15 hour period, including the normal 7-8 hour sleep period, prior to the commencement of the experiment.
- (ii) Mild dehydration. During the experiment, subjects drank only that which was necessary to quench thirst.
- (iii) Normal hydration. Throughout the experiment, subjects took their customary fluid intake.
- (iv) Overhydration. In addition to normal fluid intake, subjects were given a water load of at least 500 ml.

Diet. All subjects were on their habitual diet prior to the test, and it has been assumed that all subjects would have a standard type of dietary intake, namely about 70 g. protein and 10-12 g. sodium chloride (Manual of Nutrition, 1959). These two dietary items are specifically mentioned since they were the major items likely to affect the results to any great extent.

Collection of specimens. All urine specimens were non-catheter specimens. At the beginning of the test period, the bladder was emptied completely and the urine discarded. Accurately timed specimens were then collected at approximately hourly intervals unless otherwise stated. Blood specimens were withdrawn at some point during the test period, usually during the last collection period.

Urine examination. All urine specimens were examined for the presence of protein, sugar and blood. Protein was detected using Albustix* reagent strips and/or precipitation with 10% trichloroacetic acid; sugar using Clinistix* reagent strips or Benedict's qualitative test (Varley, 1963), and haemoglobin using Occultest* reagent tablets. The centrifuged deposit of all urine specimens was examined microscopically for the presence in abnormal amounts of pus cells, casts and red blood corpuscles.

* Ames Company, Stoke Poges, Bucks., England.

SUBJECTS.

In experiments in which tests of renal function were applied, the subjects were classified into the following groups:-

Group I. Normal controls, consisting of healthy members of the departmental staff.

Group II. Patient controls, consisting of patients with no previous history of renal disease, and no symptoms suggestive of renal dysfunction at the time of investigation. All in-patients in this group were subject to the normal hospital regime and were at rest in bed prior to the test.

The following groups all consisted of ambulant patients who were diagnosed cases of chronic renal disease, and who exhibited symptoms and/or signs consistent with renal disease at the time of investigation. All cases of "extra-renal" uraemia were excluded. No single case could be regarded as terminal renal failure.

Group III. Renal disease patients with normal blood urea concentration (20 - 40 mg. per 100 ml.).

Group IV. Renal disease patients with moderately elevated blood urea concentrations (41 - 100 mg. per 100 ml.).

Group V. Uraemic patients with blood urea concentration greater than 100 mg. per 100 ml.

Environmental conditions. Subjects in group I continued with their normal working routine throughout the tests. Patients in groups II, III, IV and V were brought to a patient's room in the Department to allow a more careful control of conditions throughout the test. Patients remained

seated during the 3-hour test period, usually reading. Room temperature was maintained at $20^{\circ} - 22^{\circ}$.

All subjects were requested to restrict their fluid intake to one cupful (100 - 150 ml.) during the 1 - 2 hour period immediately preceding the test, and were maintained under mild dehydration during the 3-hour test period. Subjects were permitted to smoke during the test period.

STATISTICAL METHODS.

(1) Calculation of mean and standard deviation.

The mean and standard deviation of a given group of results were calculated using the formulae:-

$$\text{Mean: } \bar{x} = \frac{\sum x}{n}, \quad \text{Standard deviation: } s = \pm \sqrt{\frac{\sum x^2 - \frac{(\sum x)^2}{n}}{n - 1}}$$

where x represents the individual results in a group
and n represents the number of results in a group.

(2) Student's t-test.

This test was used to compare the means of unpaired samples using the formulae:-

$$t = \frac{\bar{x} - \bar{y} \sqrt{n_x n_y}}{s \sqrt{n_x + n_y}} \quad \text{where } s = \pm \sqrt{\frac{\sum x^2 - \frac{(\sum x)^2}{n_x} + \sum y^2 - \frac{(\sum y)^2}{n_y}}{n_x + n_y - 2}}$$

\bar{x} and \bar{y} are the mean values of the two groups,

n_x and n_y are the number of samples in each group and

$n_x + n_y - 2$ represents the degrees of freedom.

(3) Method of least squares.

The equation to represent the best straight line to fit a given set of data was found using the formula:-

$$y - \bar{y} = \frac{S_{xy} - n\bar{x}\bar{y}}{S_x^2 - n(\bar{x})^2} (x - \bar{x})$$

where x and y represent the two quantities related by the straight line, and n = the number of pairs of values x, y.

(4) Correlation Coefficient (r)

This was used to establish the degree of correlation between the variables x and y.

$$r^2 = \frac{S(x'y')}{S_x^2 - \frac{(S_x)^2}{n}} \times \frac{S(x'y')}{S_y^2 - \frac{(S_y)^2}{n}}$$

$$\text{where } S(x'y') = S_{xy} - \frac{S_x S_y}{n}$$

and n = number of pairs of values x, y.

(5) Significance of correlation coefficient.

The significance of the correlation coefficient (r) was tested using the formula:-

$$t = \frac{r \sqrt{n - 2}}{\sqrt{1 - r^2}}$$

where $n - 2$ represents the degrees of freedom corresponding to n pairs of values x, y .

P values corresponding to the t values were obtained from Documenta Geigy Scientific Tables (1962) for the appropriate degrees of freedom. Results were only considered significant when the calculated value of t exceeded the value of t at the 5% probability level ($P = 0.05$).

RESULTS.

URINE OSMOLALITY IN NORMAL SUBJECTS.

Urine osmolality was studied under different states of hydration, and under conditions of osmotic diuresis, to establish the limits within which osmolality could be used as a measure of normal renal concentrating capacity.

Urine osmolality after dehydration as an index of renal concentrating capacity.

Urine osmolality was measured in 14 subjects (7 males and 7 females), following a 14 - 15 hour dehydration period. During the 13 - 14th hour of the period, the bladder was emptied and urine discarded. Approximately one hour later, a urine specimen was collected, and urine osmolality measured. This procedure was repeated on the same subjects 10 - 14 days later. The experiment was similar to that carried out by Jacobson, Levy, Kaufman, Gallinek and Donnelly (1962) and De Leon, Dreifus and Bellet (1960). In both of these studies a similar length of dehydration period was used, and the urine osmolality at the end of the dehydration period was taken as a measure of renal concentrating ability. Urine volume was not measured either in the present experiment or in the studies mentioned above. The results of the experiment on both days are shown in Table III.

The mean value and standard deviation were similar on the two occasions, indicating that for this group of subjects as a whole, the results were reproducible. However in a few individuals there was a marked difference between the two results, e.g. subjects 4, 11, and 12 (difference greater than 200 mOsm. per Kg. water). The most disturbing feature of the results

was the considerable variation between subjects. This is shown by the wide range of values obtained within the group on both occasions. This variation was not accounted for by difference in sex, since there was no significant difference between results on males and females. 7 of the subjects were smokers, but there was no significant difference between results on smokers and non-smokers.

The effects of dehydration were very variable, even in normal subjects under similar conditions. The aim of the experiment was to obtain an index of normal renal function which could be used to distinguish subjects with normal renal function from subjects with chronic renal disease. The variation in normals was such that no precise index of normal renal function could be obtained under these conditions.

Further studies on the same normal subjects showed that long periods of dehydration were not essential for the production of a high urine osmolality. Many of the subjects could attain and exceed the values obtained after 14 - 15 hour dehydration, under conditions of normal hydration or only mild dehydration. This is shown in Table IV for 3 subjects. The values represented osmolality measurements on successive hourly specimens of urine.

The data in Table IV compares the urine osmolality produced after dehydration with that attained by the same subjects throughout the day, under normal hydration or only mild dehydration. All 3 subjects were able to achieve highly concentrated urine specimens without long periods of prior dehydration. In addition, the results obtained during the day were more consistent for any one subject than those obtained following a 14 - 15 hour dehydration period. As a result of this finding, a further investigation of changes in urine

osmolality and urine flow rate was carried out on normal subjects under different states of hydration.

The relationship between solute excretion and urine flow rate under different states of hydration.

Changes in solute output per unit time (mOsm. per 60 min.) and urine flow rate (ml. per 60 min.) were investigated over a wide range of urine flow rates. The state of hydration of the subjects varied from mild dehydration to overhydration. Accurately timed specimens were collected from all subjects at approximately hourly intervals. Urine volume and osmolality were measured for each specimen and the corresponding urine flow rate and solute excretion rate were calculated. 250 specimens were obtained from 10 normal subjects. The relationship between solute excretion, and urine flow rate over a wide range of urine flow rates is shown in Fig. 5.

The data fell into two groups (A and B), distinguished by the fact that in group A a linear relationship existed between solute excretion rate and urine flow rate, and in group B there was no apparent relationship between the two parameters. The transition from group A to group B occurred experimentally at a urine flow rate of 75 ml. per 60 min.

TABLE III.

URINE OSMOLALITY AFTER DEHYDRATION.

<u>Urine osmolality (mOsm./Kg. water)</u>			
<u>Subject</u>	<u>Sex</u>	<u>1st Experiment</u>	<u>2nd Experiment</u>
1	F	585	713
2	*F	701	791
3	M	748	902
4	*M	770	1,206
5	F	773	793
6	F	823	922
7	F	847	873
8	*F	859	786
9	*M	972	911
10	*F	979	978
11	M	1,002	1,204
12	M	1,064	839
13	*M	1,073	1,021
14	*M	1,104	1,001
		<u>Mean</u> : 879	<u>Mean</u> : 924
		<u>S.D.</u> : ± 157	<u>S.D.</u> : ± 145

* Smokers.

TABLE IV.

URINE OSMOLALITY AFTER (a) 14 - 15 HOUR DEHYDRATION COMPARED WITH

(b) NORMAL HYDRATION OR MILD DEHYDRATION.

<u>Subject</u>	<u>Urine osmolality (mOsm./Kg. water)</u>	
	(a)	(b)
1	585) 713)	- 783, 739, 792
3	748) 902)	- 866, 957, 871
4	770) 1,206)	- 951, 970, 972

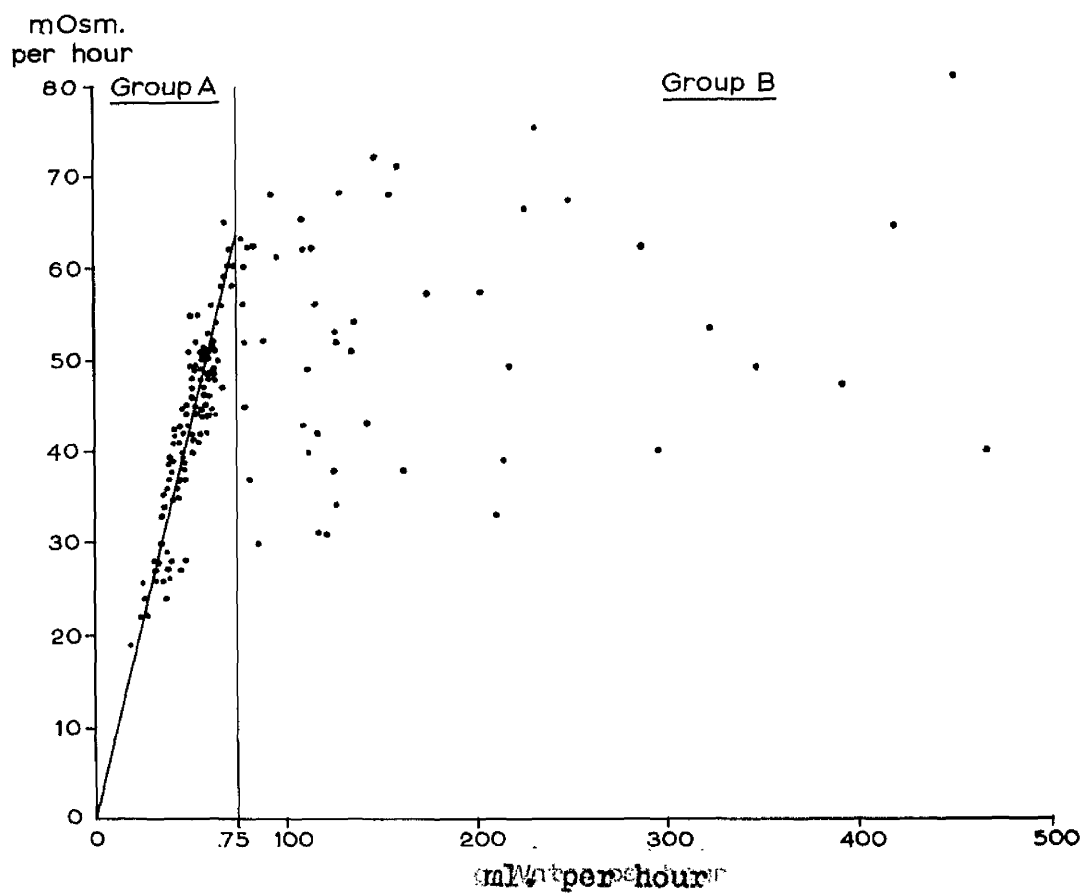


FIGURE 5. THE RELATIONSHIP BETWEEN SOLUTE EXCRETION AND URINE FLOW RATE UNDER DIFFERENT STATES OF HYDRATION.

Group A. (200 points): In this group, the urine flow rate varied from 14 - 75 ml. per 60 min., and the solute excretion from 15 - 61 mOsm. per 60 min. Fig. 6 shows only the data for group A. Using the method of least squares, the equation to represent the best straight line through this data was:

$$y = 0.719 x + 6.72 \dots\dots\dots (2)$$

where x = urine flow rate (ml. per 60 min.)

y = solute excretion (mOsm. per 60 min.)

The theoretical line to represent this equation is shown in Figs. 5 and 6. The correlation coefficient (r) for the data was 0.94. It was more convenient to express equation (2) in the form,

$$\text{Osmolality} = 719 + \frac{6720}{x} \text{ mOsm. per Kg. water} \dots\dots\dots (3)$$

This equation would hold for any value of x in the observed urine flow range 14 - 75 ml. per 60 min. The urine flow rates in group A were obtained under conditions of normal hydration or only mild dehydration. The maximal observed osmolality was 1200 mOsm. per Kg. water, and the minimal value was 809 mOsm. per Kg. water.

Provided the urine flow rate lies within the required range, it should be possible using equation (3) to calculate the theoretical osmolality corresponding to a given urine flow rate. This value could then be compared with the observed urine osmolality. In normal subjects, the theoretical and observed osmolality compare closely with one another, as a result of the distribution of normal data in group A.

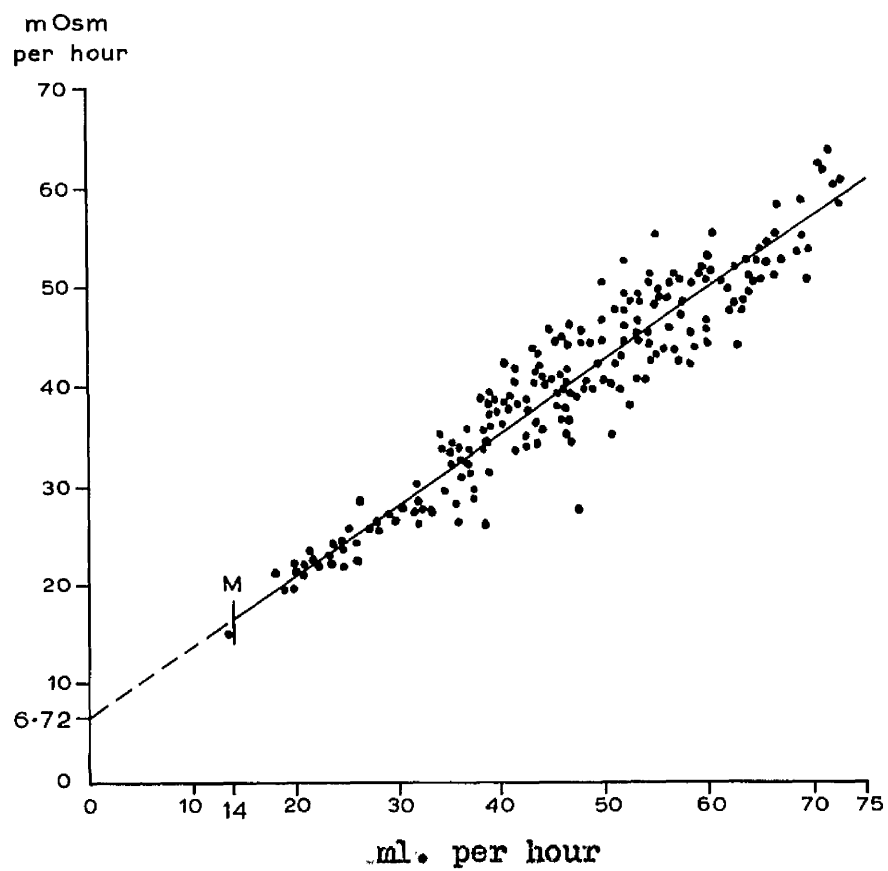


FIGURE 6.

RELATIONSHIP BETWEEN SOLUTE EXCRETION AND URINE FLOW

RATE IN GROUP A.

The relationship between observed and theoretical osmolality could be used as an index of renal function, and any gross deviation between observed and theoretical osmolality could be taken as an indication of renal dysfunction.

Group B (50 points): In this group, the urine flow rate ranged from 78 - 465 ml. per 60 min., the solute excretion rate from 30 - 81 mOsm. per 60 min., and the urine osmolality from 808 - 87 mOsm. per Kg. water. The transition from a linear relationship (group A) to a completely random scatter occurred very abruptly; at urine flow rates only slightly greater than 75 ml. per 60 min. wide variation in urine osmolality was apparent, e.g. in the range 75 - 100 ml. per 60 min. the observed osmolality varied from 808 - 346 mOsm. per Kg. water.

Such wide variation in normal subjects over a relatively narrow range of urine flow rates indicated that little reliance could be placed on the measurement of urine osmolality as an indication of renal concentrating ability unless the urine flow rate was known. It seemed probable that some of the low osmolality values observed after dehydration (Table III, p. 54) may have represented specimens with urine flow rates greater than 75 ml. per 60 min.

The use of the relationship in equation (3) as the basis of a test of renal function has one obvious limitation, namely that it would be applicable over only a relatively narrow range of urine flow rates. Many renal disease subjects have a marked polyuria, and would require considerably more dehydration than normals to attain urine flow rates of less than 75 ml. per 60 min. For this reason, additional data was obtained at urine flow rates greater than

75 ml. per 60 min. and combined with the data in group A, in an attempt to define the relationship which governed urine osmolality at urine flow rates above 75 ml. per 60 min.

Fig. 7 shows the relationship between urine osmolality and urine flow rate for a series of 100 specimens obtained from 12 normal subjects. The curve was in the form of a hyperbola, and the general equation to represent such a curve is:

$$y = \frac{a}{x} + b \dots\dots\dots(4) \text{ where } a \text{ and } b \text{ are constants.}$$

By choosing selected points on the curve, the constants a and b can be evaluated. In practice, with a hyperbolic curve, it is better to replot $1/y$ against x, and hence obtain a straight line (Defares and Sneddon, 1960).

The equation to represent this straight line was:

$$y = 0.02 x + 0.34 \dots\dots(5) \text{ where } y = \frac{1000}{\text{osmolality}}$$

$$x = \text{urine flow rate ml. per 60 min.}$$

Fig. 8 shows the relationship between the reciprocal of the urine osmolality and the urine flow rate.

There was considerable scatter of results, and consequently the theoretical osmolality for a known urine flow rate could not be calculated with any confidence from equation (5). The results were of interest, however, in that they showed a definite relationship between urine osmolality and urine flow rate, in contrast to the random scatter when solute excretion rates were plotted against urine flow rate (Fig. 5, group B, p. 56).

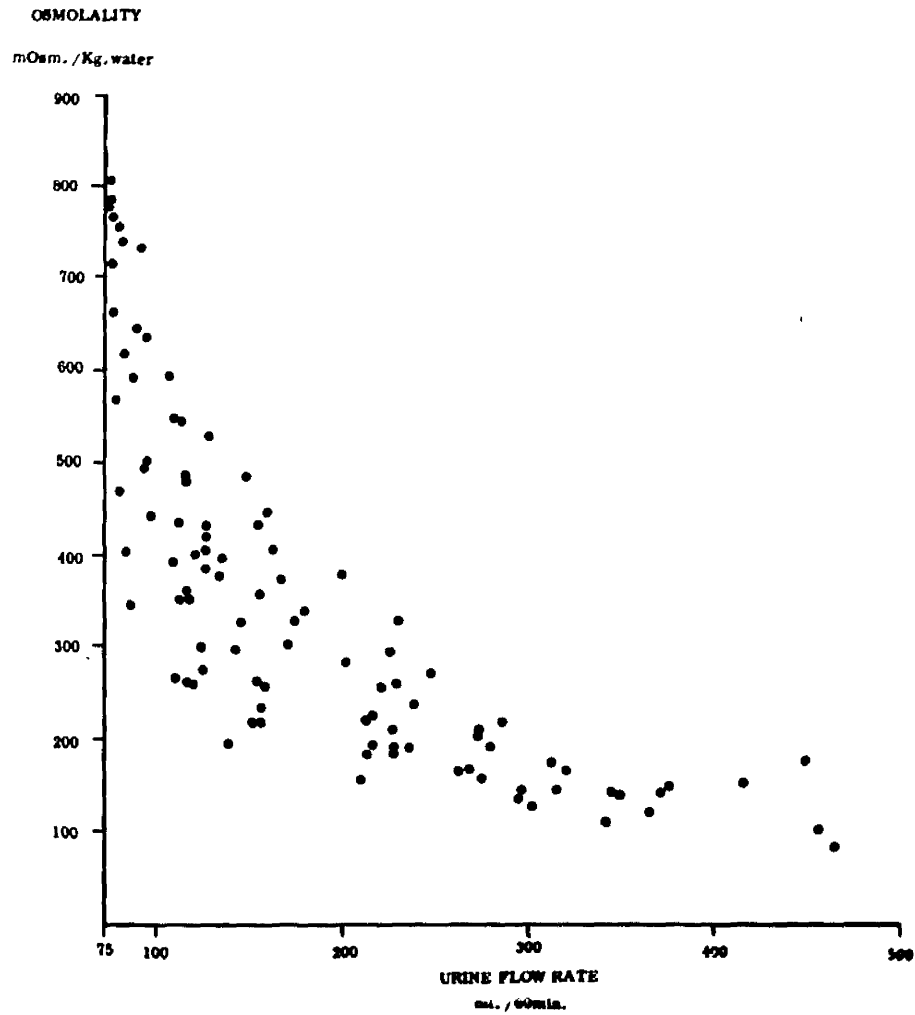


FIGURE 7.

URINE OSMOLALITY AT URINE FLOW RATES GREATER THAN

75 ML. PER 60 MIN.

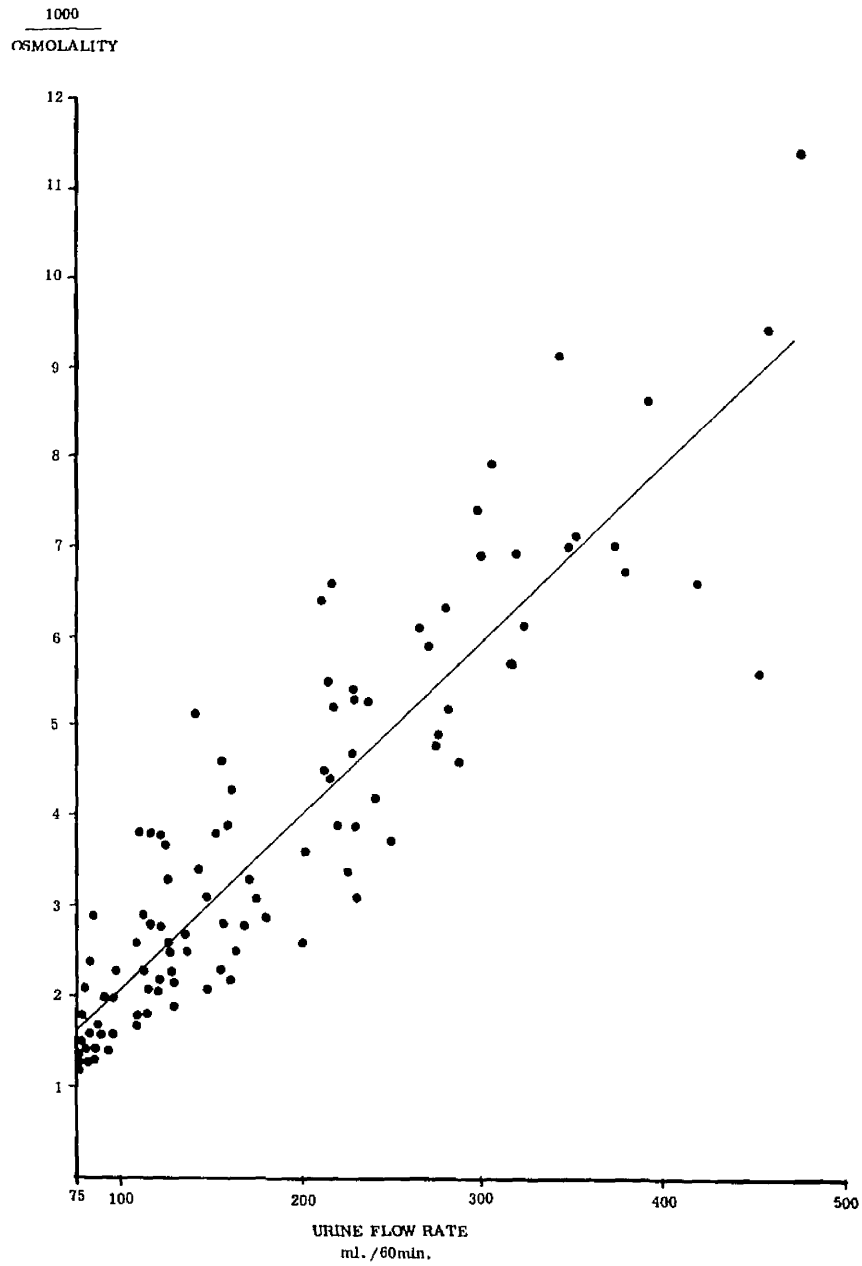


FIGURE 8.

RECIPROCAL PLOT OF URINE OSMOLALITY.

Water diuresis in normal subjects.

The fundamental cause of the scatter in Fig. 8 was unknown. It is well known, however, that during the onset of a water diuresis rapid changes occur in both urine flow rate and solute excretion, and it seemed likely that much of the scatter might have been due to this transition from normal hydration to a water diuresis.

Following a water load, e.g. 500 ml., an increase in urine flow rate generally occurs after 30 min. and reaches a maximum 1 - 2 hours after the ingestion of the water (Pitts, 1963b). The time of maximal diuresis depends mainly on the magnitude of the load, and the rate of absorption. During the period between the onset and the peak of the water diuresis, the urine flow rate will be changing very rapidly. Consequently, specimens collected during this phase over the normal one-hour collection period must represent "mixed specimens" (i.e. a mixture of concentrated and dilute urine).

Urine specimens were collected at 15 minute intervals during the onset of and recovery from a water diuresis, to establish the changes which would occur during a one-hour period. The results obtained from 2 normal subjects are shown in Table V. If the specimens had been collected as a single one-hour specimen, the urine flow rate and osmolality would have been represented by the mean values in Table V, assuming that neither water nor solute is reabsorbed from the bladder in appreciable amounts during a one-hour period. The mean value was not representative of the changes which occurred within the one-hour period.

All specimens in Fig. 8 were collected over a one-hour period and many of them represented the development of, or recovery from, a water diuresis.

The Changes in urine flow rate and osmolality during normal hydration.

Changes in urine flow rate and osmolality during normal hydration.

In contrast to the changes during a water diuresis, only minimal changes occur under normal hydration. Table VI shows urine osmolality and urine flow rate for 2 normal subjects, when specimens were collected at 15 minute intervals under normal hydration. The mean value obtained for the one-hour collection period reflected the changes which occurred in urine volume and osmolality within that period.

TABLE V.

CHANGES IN URINE FLOW RATE AND OSMOLALITY DURING A WATER DIURESIS.

<u>Subject A:</u>	<u>Collection period</u> (15 min.)	1.	2.	3.	4.	<u>Mean</u> <u>1-hour value</u>
<u>Onset of a</u>	Urine volume (ml.)	7 $\frac{1}{2}$	13	51	147	219
<u>water diuresis</u>	Osmolality (mOsm./ Kg. water)	932	729	211	73	174

Subject B:

<u>Recovery from</u>	Urine volume (ml.)	130	81	28	11	250
<u>water diuresis</u>	Osmolality (mOsm./ Kg. water)	101	148	333	625	166

TABLE VI.

CHANGES IN URINE FLOW RATE AND OSMOLALITY
DURING NORMAL HYDRATION.

<u>Subject A:</u>	<u>Collection period</u> (15 min.)	1.	2.	3.	4.	<u>Mean</u> <u>1-hour value</u>
	Urine volume (ml.)	10 $\frac{1}{2}$	12 $\frac{1}{2}$	8	8 $\frac{1}{2}$	39 $\frac{1}{2}$
	Osmolality (mOsm./ Kg. water)	910	738	893	895	851
<u>Subject B:</u>	Urine volume (ml.)	12 $\frac{1}{2}$	13	12 $\frac{1}{2}$	13	51
	Osmolality (mOsm./ Kg. water)	910	953	905	928	918

Patterns of solute excretion and urine flow rate during a water diuresis.

The transition from normal hydration to a water diuresis and the return to normal hydration was studied in 4 normal subjects using shorter collection periods of 15 minutes, to obtain a more accurate representation of the diuresis pattern. Diuresis was induced by a water load of 500 ml. taken orally over a 15 minute period. The typical pattern obtained for one of the subjects is illustrated in Fig. 9.

The specimens before the onset of the diuresis may be regarded as control periods during which only slight variation in solute output and urine flow rate occurred. Throughout the diuresis, the urine flow rate followed the expected pattern, with a rapid increase in flow rate over a short period, reaching a sharp maximum, and a rapid return to low urine flow rates similar to those in the pre-diuresis period.

Initially, the solute excretion rate increased with the urine flow rate, but the peak solute output occurred before the peak urine flow rate, and then decreased while the urine flow rate was still rising. This was a constant finding in the subjects studied, and the separation in time of the two peaks varied from 30 - 45 minutes. As a result, the patterns of solute excretion and urine flow rate were "out of phase" over this region. With specimens collected over a one-hour period, it is unlikely that this difference in pattern would have been detected.

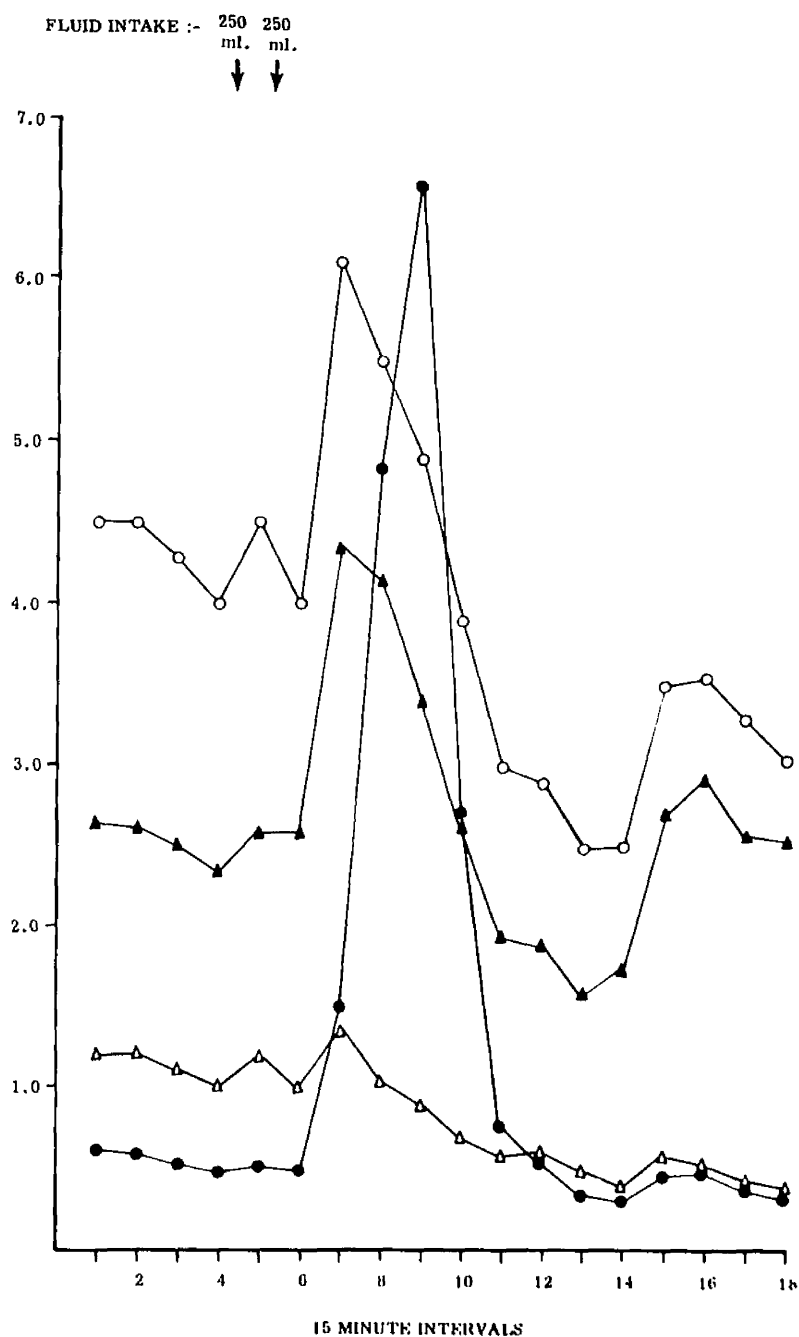


FIGURE 9.

PATTERNS OF SOLUTE EXCRETION AND URINE FLOW RATE

DURING A WATER DIURESIS.

- | | | | |
|-------|---------------------------------------|-------|---------------------------|
| ● — ● | urine flow rate (ml./min.) | △ — △ | chloride (meq./min. x 10) |
| ○ — ○ | solute excretion
(mOsm./min. x 10) | ▲ — ▲ | urea (mg./min. x 0.25) |

The solute excretion rose from 0.43 mOsm. per min. in the pre-diuresis period to 0.62 mOsm. per min. at the solute peak, representing an increase of 44%. Estimation of urea and chloride in each specimen indicated that the excretion pattern of these solutes reflected that of the total solute pattern, although the absolute increase in the individual solutes differed considerably. The mean urea excretion rate was 10.0 mg. per min. in the pre-diuresis period, and it rose to 17.5 mg. per min. representing an increase of 75%. The increase in chloride excretion was considerably less, rising from 0.10 meq. per min. in the pre-diuresis period to 0.13 meq. per min. at the peak. This represented an increase of only 33%. Urea contributed the major increase in solute excretion observed at the onset of the water diuresis.

During mild dehydration, the low urine flow rate imposes a limit on the amount of solute which is excreted. It is known that during anti-diuresis, urea accumulates within the kidney (Berliner et al. 1958). This urea is thought to come from the collecting duct which in the presence of vasopressin has been shown to have an increased permeability towards urea (Jaenike, 1961). During antidiuresis, other solutes e.g. sodium salts are used in establishing an osmotic gradient within the medulla, to allow final concentration of the urine in the collecting ducts (Berliner et al. 1958). The brief increase in solute excretion and the solute peak observed at the onset of a water diuresis could be regarded as a "wash-out" effect by which this accumulation of solute is removed.

Successive periods of diuresis were induced in one subject to establish if the increase in solute excretion occurred at the onset of the second and

subsequent diuresis periods. The results are shown in Fig. 10. With each diuresis period, a discrete increase in solute excretion and a solute peak occurred before the maximal urine flow rate. The absolute value of the peak varied with each diuresis, but there was no evidence of a marked decrease in solute excretion peak with successive diuresis periods.

Concentrating ability in post-diuresis specimens.

During the post-diuresis period (Fig. 9, p. 68) when urine flow rates had returned to basal levels, the solute output was less than in the pre-diuresis period. The mean pre-diuresis solute output was 0.43 mOsm. per min. compared with 0.27 mOsm. per min. in the immediate post-diuresis period. Since the post-diuresis specimens had urine flow rates less than 75 ml. per 60 min., it was possible to calculate the theoretical osmolality for each specimen from equation (3) p. 57. The ratio observed: theoretical osmolality expressed as a percentage was taken as a measure of concentrating ability. Table VII shows the concentrating ability of pre- and post-diuresis specimens.

In the first post-diuresis period, the concentrating ability was markedly lower in all subjects. There was a gradual return to normal concentrating ability within 1 - 2 hours after the diuresis. This illustrates the need for a careful control of fluid intake both prior to and during any test of renal concentrating ability, since even normal subjects have a low concentrating ability in the immediate post-diuresis period.

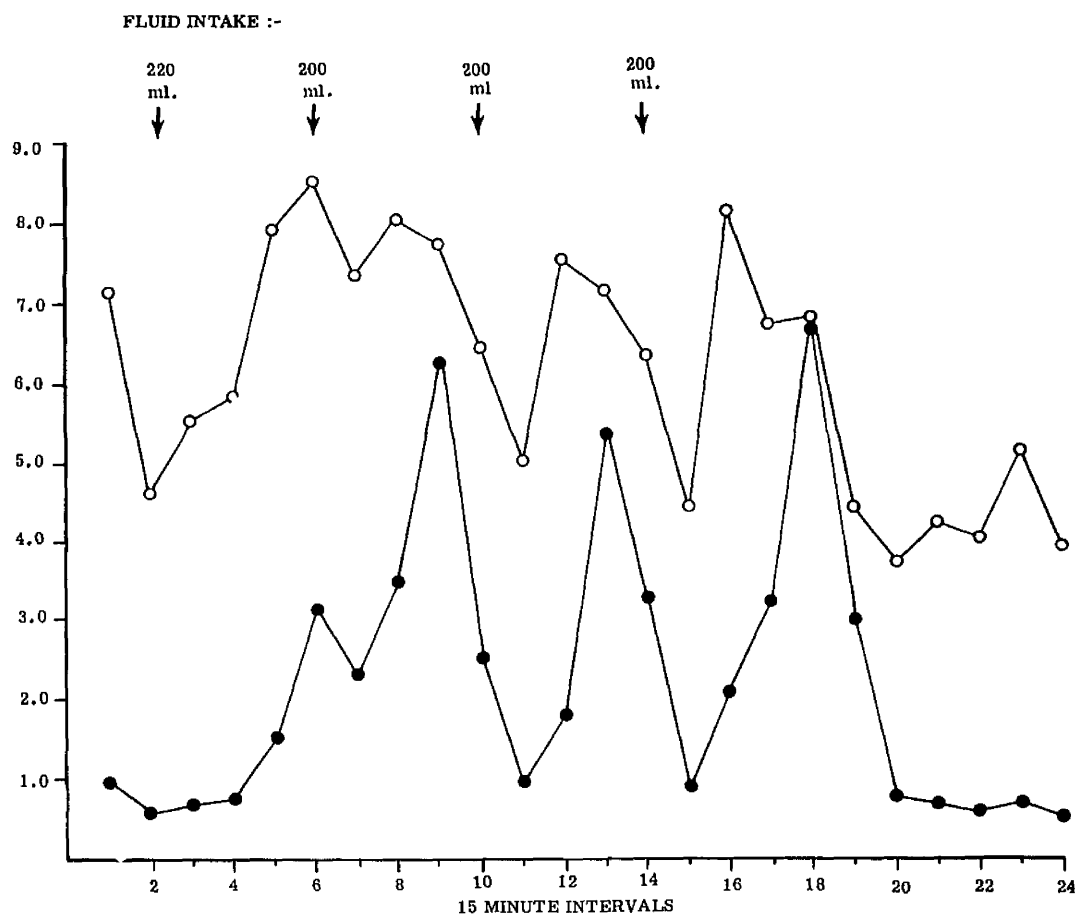


FIGURE 10. SUCCESSIVE DIURESIS PERIODS.

● — ● urine flow rate (ml./min.)
 ○ — ○ solute excretion (mOsm./min. x 10)

TABLE VII.

CONCENTRATING ABILITY IN PRE-DIURESIS AND POST-DIURESIS SPECIMENS.

<u>Subject</u>	<u>Pre-diuresis concentrating ability</u>			<u>Post-diuresis concentrating ability</u>		
	1.	2.	3.	1.	2.	3.
1.	95%	98%	100%	63%	80%	82%
2.	89%	93%	89%	56%	71%	78%
3.	96%	93%	89%	74%	74%	70%
4.	116%	108%	111%	74%	93%	88%

Solute induced diuresis.

The effect of a solute induced diuresis as opposed to a water diuresis was studied to assess the changes in urine flow rate and solute output which would accompany such a diuresis. The major urinary solutes which contribute to urine osmolality are urea, sodium and chloride. An osmotic diuresis was induced in a normal subject using urea.

- (i) Urea. Acute urea loading produces a mild diuretic effect. After several control periods, during which urine volume and osmolality were measured, the subject ingested 10 g. urea in 100 ml. water. As a result of this increased osmotic load, urine flow rate increased above 75 ml. per 60 min., and reached a maximum of 136 ml. per 60 min. Fig. 11 shows the relationship between the rate of solute output (mOsm. per 60 min.) and urine flow rate during this osmotic diuresis. The linear relationship persisted, even at urine flow rates greater than 75 ml. per 60 min., in contrast to the results obtained during a water diuresis. The reason for this became apparent when solute excretion patterns and urine flow rate were plotted on the appropriate time scale (Fig. 12). During a water diuresis, it was observed that the peak solute excretion rate occurred before the maximal urine flow rate (Fig. 9), but with an osmotic diuresis induced by urea, the solute excretion peak and the maximal urine flow rate coincided, so that at no time were the solute excretion and urine flow rate patterns "out of phase".

SOLUTE EXCRETION
mOsm./60min.

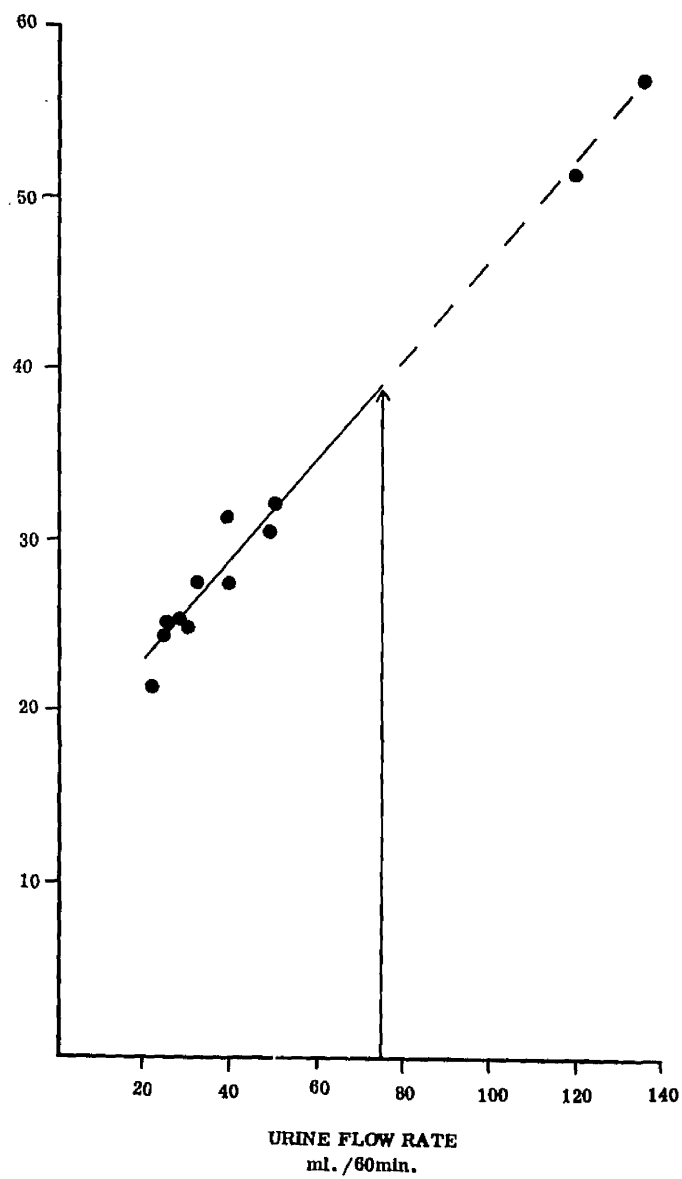


FIGURE 11. OSMOTIC DIURESIS: (1) UREA.

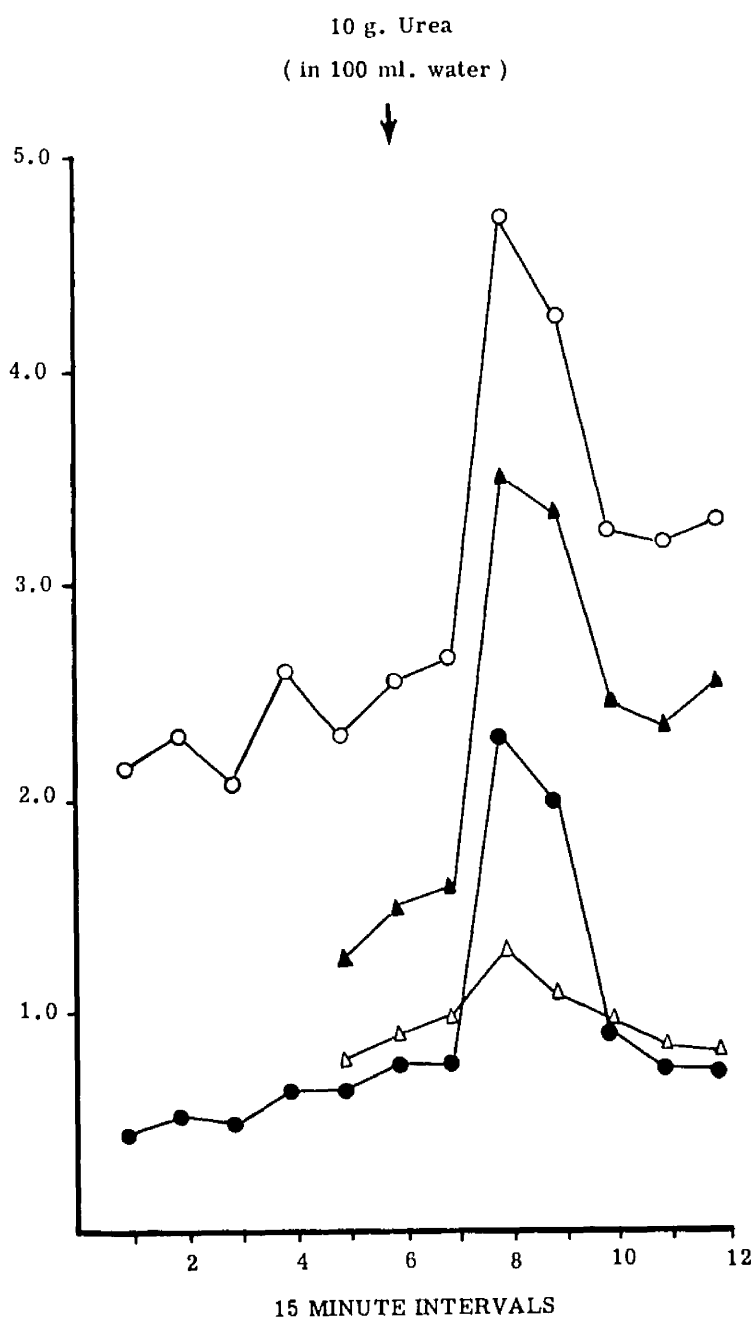


FIGURE 12. PATTERNS OF SOLUTE EXCRETION DURING OSMOTIC DIURESIS.

(1) urea induced

● —● urine flow rate (ml./min.) ▲ —▲ urea excretion (mg./min. x 0.1)
 ○ —○ solute excretion (mOsm./min. x 5) △ —△ chloride excretion (meq./min. x 10)

(ii) Frusemide* diuresis.

A diuresis was induced using Frusemide diuretic tablets in a normal subject. This diuretic induces a prompt diuresis in which there is a marked increase in the excretion of water, sodium and chloride, with only a minimal increase in potassium excretion. The main action of Frusemide is the inhibition of tubular reabsorption of sodium and chloride.

Urine specimens were collected at 30 minute intervals throughout the experiment. After several control periods, 80 mg. of Frusemide were taken in 150 ml. water. Fig. 13 shows the relationship between urine flow rate and solute excretion rate before and after Frusemide administration. The urine flow rate increased within 30 minutes and reached a maximal value of 12.9 ml. per min. The results were similar to urea-induced diuresis, with the linear relationship between solute excretion and urine flow rate continuing at urine flow rates greater than 75 ml. per 60 min. In Fig. 14, the solute excretion patterns and urine flow rates are shown during the Frusemide diuresis. As with the urea diuresis, the maximal solute excretion and urine flow rate coincided, and the patterns were comparable throughout. One interesting feature of the Frusemide diuresis was the considerable increase in urea excretion which accompanied the increase in sodium and chloride excretion.

The results indicated that much of the scatter previously observed at urine flow rates greater than 75 ml. per 60 min. was associated with the onset of a water diuresis. When diuresis was induced by an increased solute load, the linear relationship between solute excretion and urine flow rate continued even at flow rates greater than 75 ml. per 60 min.

* Lasix, Hoechst, Frankfurt, Germany.

SOLUTE EXCRETION

mOsm. /min.

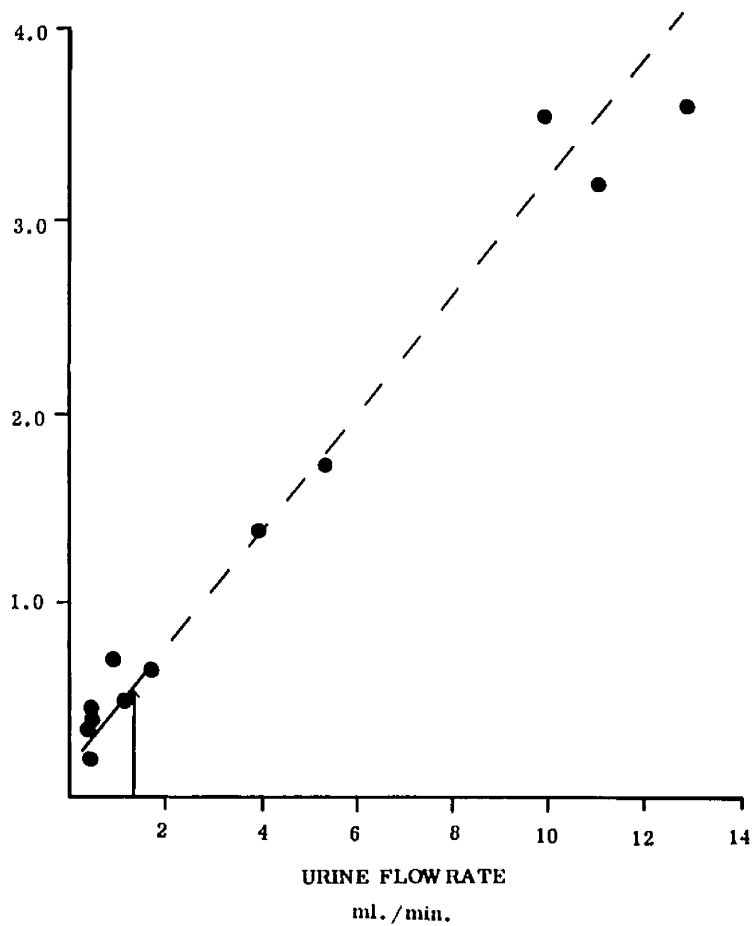


FIGURE 13. (11) FRUSEMIDE INDUCED DIURESIS.

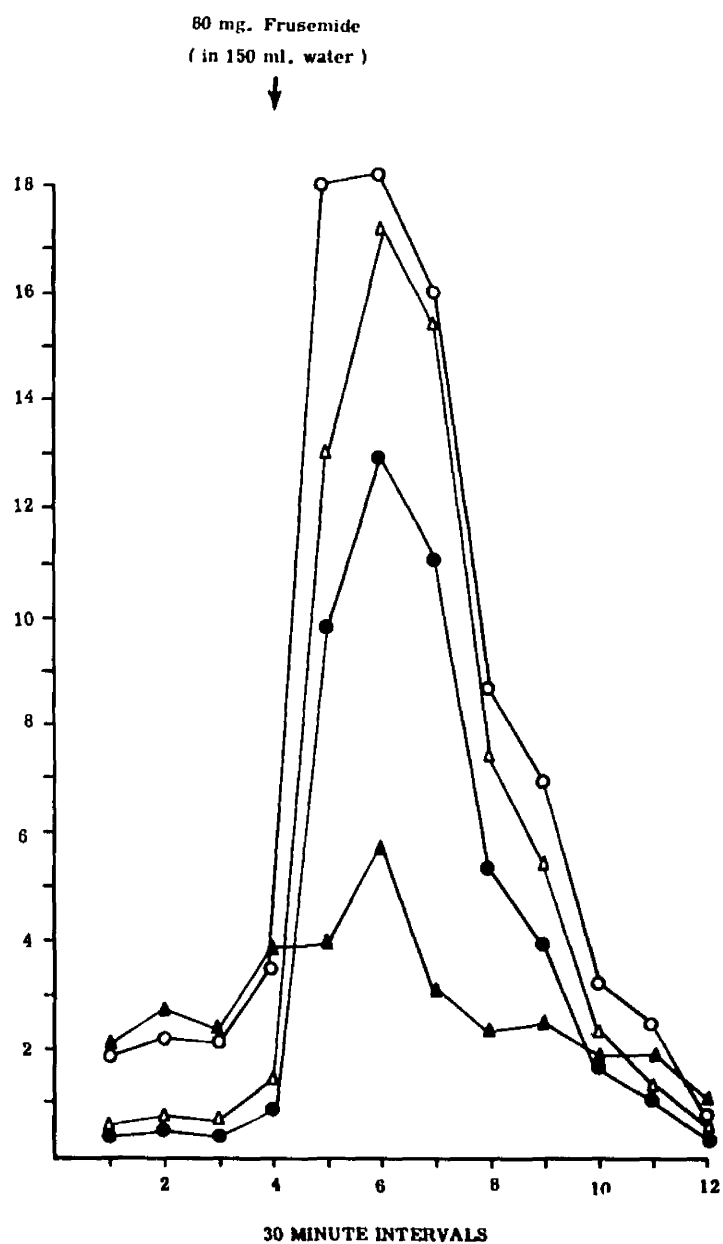


FIGURE 14. PATTERNS OF SOLUTE EXCRETION DURING DIURESIS.

(ii) Frusemide induced diuresis

- | | |
|---|---|
| ● — ● Urine flow rate (ml./min.) | ▲ — ▲ Urea excretion (mg./min. x 0.2) |
| ○ — ○ Solute excretion (mOsm./min. x 5) | Δ — Δ Chloride excretion (meq./min. x 10) |

Contribution of urea and electrolyte to urine osmolality at urine flow rates less than 75 ml. per 60 min.

The contribution of urea and electrolyte to urine osmolality was estimated for a series of normal control subjects. It was hoped by means of this assessment to distinguish between two situations,

- (i) Lowered total solute output due to diminished urea excretion
- (ii) Lowered total solute output due to diminished electrolyte excretion.

Since urea is a non-ionic solute, the osmolality due to urea can be calculated directly from the urea concentration. Urine conductivity was used as a measure of total electrolyte concentration. The osmolality contributed by this electrolyte was then calculated using the calibration graph shown in Fig. 4, p. 42.

For 17 normal subjects, the percentage of the osmolality due to urea ranged from 28 - 61% and that due to total electrolyte from 38 - 67%. Collectively, the contribution of urea and electrolyte in any one specimen was relatively constant and ranged from 88 - 99% for the entire group.

Figure 15 shows the relationship between urea excretion (mOsm. per 60 min.) and urine flow rate for the 17 subjects. Over this range of urine flow rates, changes in urea excretion were small in comparison with changes in the total solute excretion.

The solid line in Fig. 15 represents the relationship between total solute excretion and urine flow rate in normals (Fig. 6, p. 58).

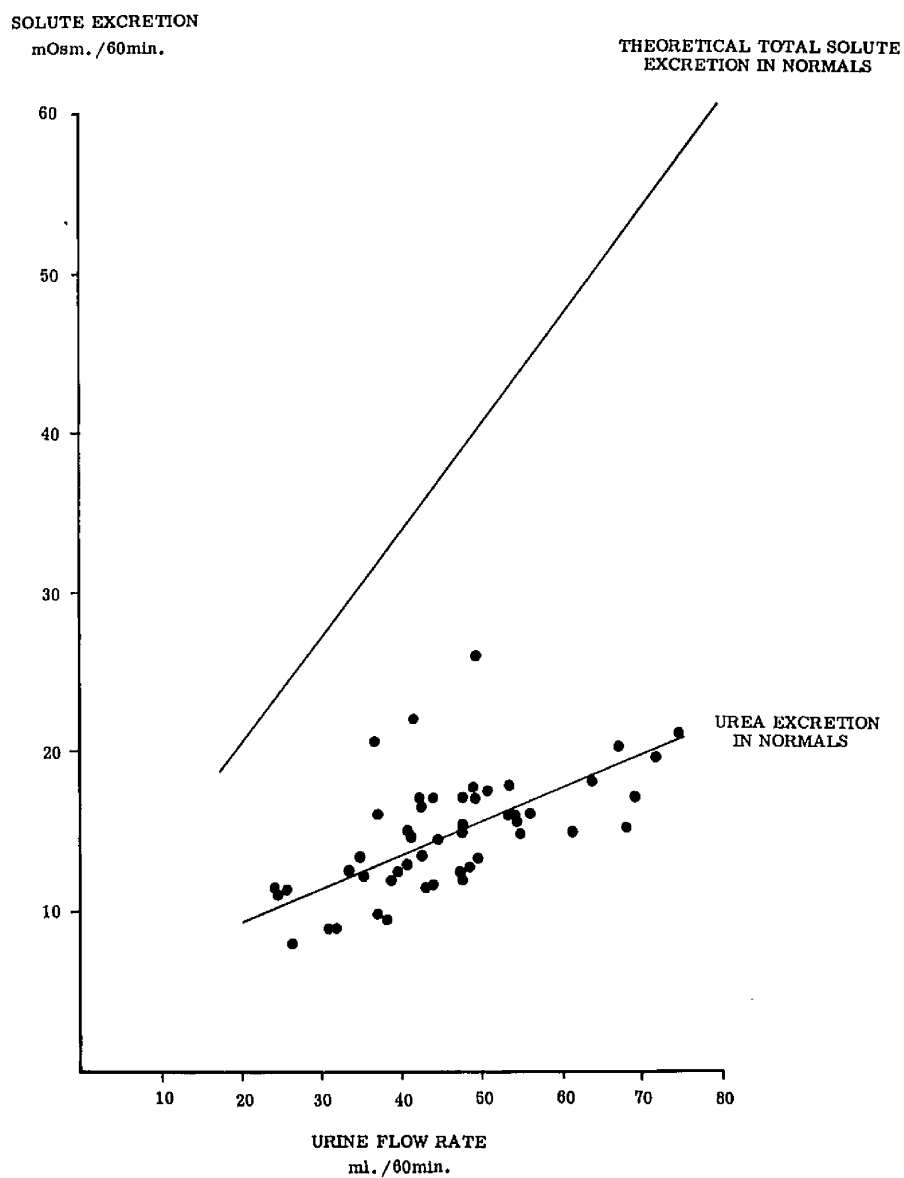


FIGURE 15. **UREA EXCRETION IN NORMAL SUBJECTS.**

Using the method of least squares, the equation to represent the data for urea excretion was:

$$y = 0.21x + 5.2 \dots\dots\dots(6)$$

where y = urea excretion (mOsm. per 60 min.)

x = urine flow rate (ml. per 60 min.)

Figure 16 shows the relationship between electrolyte excretion and urine flow rate for the same subjects. The equation to represent this data was:

$$y = 0.5x + 0.4 \dots\dots\dots(7)$$

where y = electrolyte excretion (mOsm. per 60 min.)

x = urine flow rate (ml. per 60 min.)

The slope of this line was considerably different from that for urea excretion. The range of electrolyte excretion varied much more than urea excretion over the range of urine flow rates 14 - 75 ml. per 60 min. Comparison with the line representing total solute excretion showed electrolyte excretion accounted for the major part of the increase in total solute excretion over the range 14 - 75 ml. per 60 min.

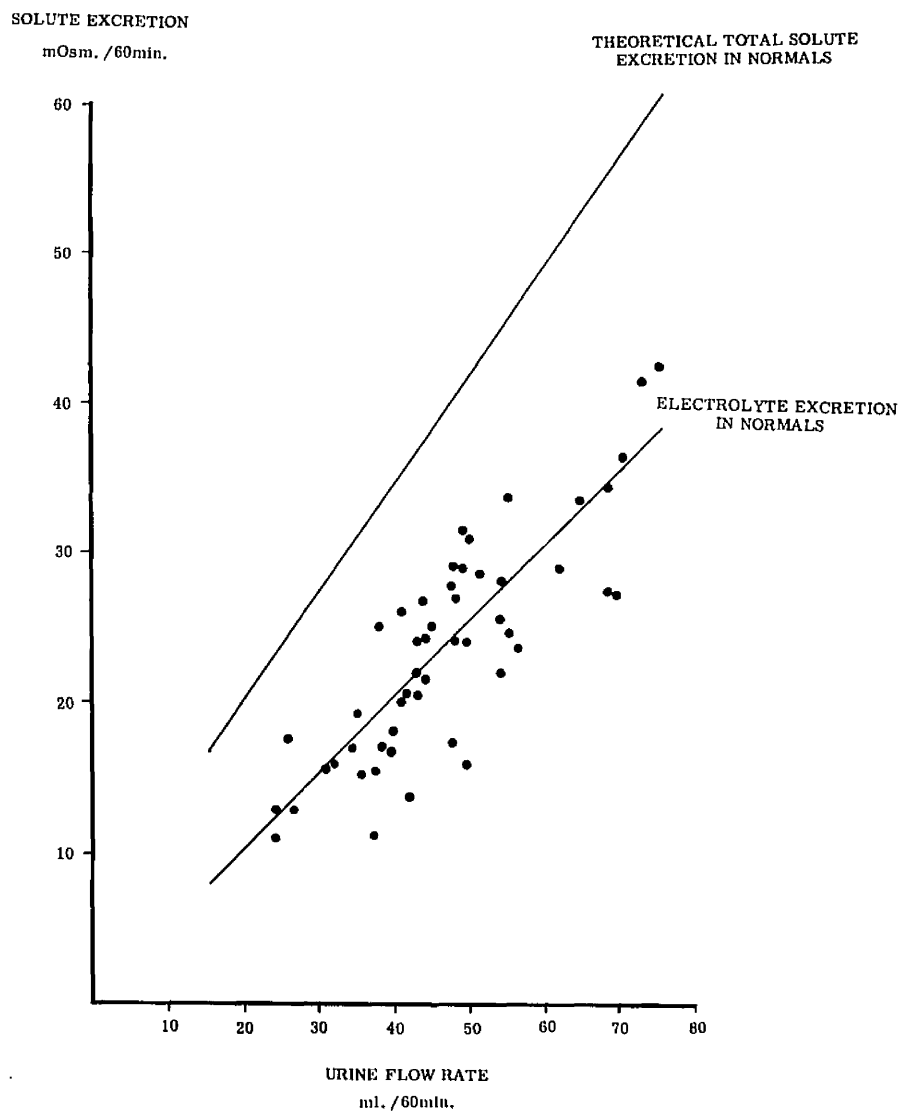


FIGURE 16. ELECTROLYTE EXCRETION IN NORMAL SUBJECTS.

A TEST OF RENAL FUNCTION BASED ON URINE OSMOLALITY.

The previous results have been concerned with the variation in urine osmolality under different states of hydration, and the aim has been to define more clearly the limits within which urine osmolality reflects the concentrating ability of the kidney. The most significant finding in normals was that a linear relationship existed between solute excretion and urine flow rate in the range 14 - 75 ml. per 60 min. The correlation between these two factors was sufficiently good ($r = 0.94$) to permit the use of this relationship as a means of assessing renal concentrating ability.

By means of the equation relating solute excretion and urine flow rate (equation 3, p. 57), the theoretical osmolality corresponding to a particular urine flow rate could be calculated, and this could then be compared with the observed osmolality. The ratio observed : theoretical osmolality expressed as a percentage gave a measure of renal concentrating ability, and hence of renal function. The results in this section are concerned with the application of this test to normal subjects, control patients, chronic renal disease patients, and patients with various abnormal clinical conditions.

Conditions:- 3 urine specimens were collected at hourly intervals from each subject. Fluid intake was normal prior to the test, with the proviso that during the 2-hour period immediately preceding the test, fluid intake was restricted to one cupful of fluid (approximately 100 - 150 ml.). During the 3-hour test period, fluids were withheld unless the subject complained

of thirst, in which case, the minimal amount of fluid necessary to quench thirst was given. In most cases, this mild dehydration was sufficient to keep the urine flow rate below 75 ml. per 60 min.

Group I - Normal control subjects.

This group consisted of 11 normal subjects. Urine osmolality and urine volumes were measured for each specimen. From the urine flow rate, the theoretical osmolality was calculated for each specimen and hence the concentrating ability.

With any new test of renal function, the reliability of the results can be assessed only by comparison with one of the standard tests of renal function applied to the same subjects. The standard of reference chosen was the Van Slyke urea clearance (Moller et al. 1928). This test is widely accepted as an indication of general renal function. Urea handling by the kidney depends both on glomerular filtration and tubular reabsorption, and this is by far the most common method of solute handling by the kidney.

The collection periods for urea clearance were identical with those used in the concentrating ability test, and hence both tests could be carried out on the same specimens. All clearances were standard urea clearances since the urine flow rate was less than 2.0 ml. per min. The average normal value for the standard urea clearance was taken as 54 ml. per min. (100%) and the range as 41 - 65 ml. per min. (76 - 120%), Peters and Van Slyke (1932). For both the concentrating ability test and the urea clearance test, the results were expressed as a percentage of the average normal, and consequently it was valid to compare directly the two

assessments of renal function.

In Table VIII, the results are shown for both tests on 11 normal subjects. Each value represents the mean over the 3 collection periods. For any one subject, the concentrating ability was relatively constant, and the individual values all fell within $\pm 7\%$ of the mean value. For the group as a whole, the mean concentrating ability was very close to 100%. For the urea clearance, the values were in the upper part of the normal range quoted by Van Slyke. This is probably accounted for by the cumulative effect of factors such as dietary protein, age, etc. which will inevitably differ from the original group of Van Slyke. It is of interest that the standard deviation was much less for the concentrating ability test. In the subjects studied, the concentrating ability test and the urea clearance gave a comparable index of normal renal function.

TABLE VIII.

GROUP I - CONCENTRATING ABILITY AND UREA CLEARANCE

IN NORMAL SUBJECTS.

<u>Subject</u>	<u>Blood urea</u> <u>mg./100 ml.</u>	<u>Concentrating</u> <u>ability %</u>	<u>Urea clearance</u> <u>%</u>
(Mean of 3 estimations)			
1.	25	98	129
2.	29	96	95
3.	26	106	130
4.	28	111	129
5.	24	84	103
6.	27	91	96
7.	22	103	148
8.	29	109	92
9.	34	103	95
10.	29	108	112
11.	24	112	113
		<u>Mean</u> : 102%	<u>Mean</u> : 113%
		<u>Range</u> : 84 - 112%	<u>Range</u> : 88 - 148%
		<u>S.D.</u> : ± 9	<u>S.D.</u> : ± 19

Application of the concentrating ability test to patients.

In normal subjects (Group I), the concentrating ability test gave comparable results to urea clearance values obtained simultaneously. One objection to using group I for comparison with renal disease patients was that the environmental conditions of the patients differed considerably from those of normal subjects. Most of the patients were in-patients who were subject to the normal hospital regime and were at rest in bed prior to the test. It was possible that the concentrating ability of normal subjects might alter, under these conditions. For this reason, it was considered necessary to apply the test to a group of control patients, i.e. hospital in-patients subject to the same environment as the renal disease patients, but with no previous history or evidence of renal disease. The criteria for inclusion in this control group were that at the time of investigation none of the patients had any symptoms or signs suggestive of renal disease; urine findings, blood urea and urea clearance were normal. The control patients had been admitted for a variety of ailments, none of which were likely to interfere with renal concentrating ability, e.g. no cases with bladder abnormalities or with circulatory failure were included.

Group II. Patient controls.

The group consisted of 8 patients. 4 of the patients had been admitted with an abnormal allergic reaction, but at the time of the present tests had recovered from this condition. The remaining 4 subjects were suffering from benign hypertension. The results for this group are shown in Table IX. All patients in this group were able to void urine

TABLE IX.

GROUP II - CONCENTRATING ABILITY AND UREA CLEARANCE

IN CONTROL PATIENTS.

<u>Subject</u>	<u>Blood urea mg./100 ml.</u>	<u>Concentrating ability %</u>	<u>Urea clearance %</u>
(Mean of 3 estimations)			
1	32	100	155
2	24	82	93
3	26	99	116
4	29	88	115
5	22	94	123
6	25	134	141
7	36	108	96
8	25	92	122
		<u>Mean</u> : 100%	<u>Mean</u> : 120%
		<u>Range</u> : 82 - 134%	<u>Range</u> : 93 - 155%
		<u>S.D.</u> : ± 16	<u>S.D.</u> : ± 20

complying with the test conditions. The results indicated that patients were able to achieve normal concentrating ability despite the fact that many of them were recumbent, and under different environmental conditions from the normal controls, prior to the test. Throughout the test, control patients were subjected to the same conditions as the renal disease groups with regard to activity, fluid intake and room temperature. The results were comparable to group I, and once again, the urea clearance values were considerably higher than the concentrating ability, and were distributed in the upper part of the normal range quoted by Van Slyke.

The test was then applied to chronic renal disease subjects to assess its sensitivity in the detection of renal dysfunction. Different forms of chronic renal disease were studied including glomerulonephritis, pyelonephritis, malignant hypertension, polycystic disease of the kidneys, inoperable renal calculi and disseminated lupus erythematosus with renal involvement. The extent of renal damage ranged from patients in the early stages of chronic renal disease, to patients with severe renal damage. The renal disease patients were subdivided into three groups on the basis of their blood urea concentration.

Group III. Patients with normal blood urea concentration (20 - 40 mg. per 100 ml.).

This group consisted of 13 subjects, all of whom were diagnosed cases of chronic renal disease. The diagnosis, clinical findings and urine findings for each of the patients in this group is shown in Table X.

TABLE X.EVIDENCE OF RENAL DAMAGE IN PATIENTS IN GROUP III.

<u>Subject</u>	<u>Diagnosis and clinical findings</u>	<u>Urine examination</u>					
		<u>Protein</u>	<u>Blood</u>	<u>Glucose</u>	<u>Casts</u>	<u>Pus</u>	<u>R.B.C</u>
1	Chronic nephritis	Pos.	Pos.	-	Gran.	-	+++
2	Chronic pyelonephritis	-	-	-	-	+	-
3	(Chronic pyelonephritis) (Hypertension) (Recurrent urinary tract infection)	-	-	-	-	+	-
4	(Renal damage following) (stone in Rt. kidney)	Tr.	Tr.	-	-	++	Occas
5	(Chronic pyelonephritis) (Hypertension)	-	-	-	-	++	-
6	Hypertension	Pos.	-	-	-	-	-
7	(Chronic pyelonephritis) (Hypertension) (Pernicious anaemia)	-	-	-	-	++	-
8	Malignant hypertension	-	-	-	-	-	-
9	(Chronic pyelonephritis) (Calculus in renal pelvis) (Urinary tract infection)	Tr.	Pos.	-	-	+	++
10	(Chronic nephritis) (a-gamma-globulinaemia)	Pos.	Pos.	-	-	-	++
11	Hypertension	Pos.	-	-	-	-	-
12	(Disseminated lupus) (erythematosus) (Hypertension)	Pos.	Pos.	Pos.	-	++	+
13	Disseminated lupus erythematosus	Pos.	-	-	Gran.	-	-

TABLE XI.

GROUP III - CONCENTRATING ABILITY AND UREA CLEARANCE IN RENAL DISEASE

SUBJECTS WITH NORMAL BLOOD UREA CONCENTRATION.

<u>Subject</u>	<u>Blood urea</u> <u>mg./100 ml.</u>	<u>Concentrating</u> <u>ability %</u>	<u>Urea clearance</u> <u>%</u>
(Mean of 3 estimations)			
1	28	66	61
2	40	84	81
3	35	82	77
4	29	61	61
5	29	91	88
6	25	88	103
7	25	75	72
8	21	39	53
9	21	63	88
10	40	46	41
11	26	77	95
12	40	80	79
13	39	72	45
<u>Mean</u> :		71%	<u>Mean</u> : 73%
<u>Range</u> :		39 - 91%	<u>Range</u> : 41 - 103%
<u>S.D.</u> :		± 16	<u>S.D.</u> : ± 19

The concentrating ability and urea clearance for the same patients are shown in Table XI.

Most of the subjects exhibited abnormal urine findings consistent with renal damage, and many of them represented the early stage of the disease, with only minimal functional changes as judged by both tests of renal function. In some of the patients, however, there was evidence of abnormal renal function (subjects 1, 4, 8, 9, 10, 13) and these were clinically of interest, since all subjects in this group had a normal blood urea concentration. The wide range of renal function covered by this one group showed once again that the blood urea was of no use as an index of renal function in this stage of renal disease. The usefulness of renal function tests was emphasised in the case of subject 8, in whom both blood urea and urine findings were completely normal, despite both sub-normal concentrating ability and urea clearance.

Table XI showed that for this group as a whole, both tests gave comparable results, and the mean, range and standard deviation for each test was very similar. This was in contrast to groups I and II in which the urea clearance was usually somewhat higher than the concentrating ability.

Since one of the purposes of the present work was to compare the sensitivity of the concentrating ability test with the urea clearance, any patients in whom there was a marked discrepancy between the tests were of considerable interest. Subjects 9 and 13 were in this category.

For subject 9, the mean urea clearance (88%) lay within normal limits, while the mean concentrating ability (63%) was sub-normal. In

view of the many factors which can affect renal concentrating ability, the tests were repeated on this subject after an interval of two days, to ensure that the low concentrating ability was a constant finding. The mean urea clearance was 87% and the mean concentrating ability was 62%.

The renal lesion in this subject consisted of pyelonephritis associated with an infection of the lower urinary tract, and the presence of a calculus in the renal pelvis. Pyelonephritis is restricted mainly to the medullary region of the kidney (Freedman and Beeson, 1958) in the initial stages. Consequently, functional disturbances will initially be confined to this region and hence urinary concentrating ability should be impaired. In addition, the presence of a calculus in the renal pelvis in this patient might also affect the concentrating process. There is no evidence for alteration in glomerular function in the early stages of pyelonephritis (Conick, Foos, Rubini and Guze, 1963) and so urea clearance is more likely to be normal in the early stages.

Subject 13 represented the reverse situation, in which the mean urea clearance value was considerably sub-normal (45%) compared with the mean concentrating ability (72%). The primary disease in this patient was disseminated lupus erythematosus. In this condition, renal involvement occurs frequently, and the type of renal lesion is most commonly lupus glomerulitis or lupus glomerulonephritis (Pollak, Conrad and Schwartz, 1964). The primary disturbance would be in glomerular function and therefore concentrating ability should not be impaired in the initial stages.

One interesting feature of this group was the results obtained in subjects 5, 6, and 12. The clinical diagnosis and the urine findings in

these subjects were consistent with the presence of renal disease and yet renal function as judged by both tests was within normal limits. The individual urine osmolalities for the three subjects are shown in Table XII.

Under the mildest form of fluid restriction, these subjects were able to produce what would normally be regarded as a "concentrated urine". The results demonstrate that the production of a concentrated urine does not exclude the possibility of renal disease.

Group IV. Renal disease patients with moderately elevated blood urea concentration (41 - 100 mg. per 100 ml.).

This group consisted of 9 subjects, and the results for both tests are shown in Table XIII. The urea clearance was markedly lower than the concentrating ability, in contrast to groups I and II, where it was 10 - 20% higher. The results showed that with deterioration in renal function, concentrating ability was no longer comparable with urea clearance.

In this group, subject 6 was of interest, since both the concentrating ability and the urea clearance were normal, despite the slightly raised blood urea. In addition to hypertension, this subject had an arterio-sclerotic gangrene of the right foot. The presence of a slightly raised blood urea was consistent with the increased protein catabolism accompanying such a condition, and did not reflect an impairment of renal function. The renal damage in this patient was presumably minimal, in view of the normal urea clearance and concentrating ability.

TABLE XII.

HIGH URINE OSMOLALITY IN SUBJECTS WITH RENAL DISEASE.

<u>Subject</u>	<u>Urine osmolality (mOsm./Kg. water).</u>		
5	831,	817,	892
6	709,	748,	730
12	831,	703,	793

TABLE XIII.

GROUP IV - CONCENTRATING ABILITY AND UREA CLEARANCE IN RENAL DISEASE
 PATIENTS WITH MODERATELY RAISED BLOOD UREA.

Subject	Blood urea mg./100 ml.	Concentrating ability %	Urea clearance %	
(Mean of 3 estimations)				
1	43	58	59	
2	48	62	34	
3	46	65	52	
4	54	44	22	
5	65	78	49	
6	46	92	79	
7	59	79	56	
8	62	75	41	
9	53	48	20	
<u>Mean</u> :		67%	<u>Mean</u> :	46%
<u>Range</u> :		44 - 92%	<u>Range</u> :	20 - 79%
<u>S.D.</u> :		± 16	<u>S.D.</u> :	± 19

Group V. Uraemic patients (Blood urea > 100 mg. per 100 ml.).

The results of concentrating ability and urea clearance for this group are shown in Table XIV. The small number of patients in this group was dictated by the difficulty in obtaining subjects who did not have a polyuria. In many of the uraemic patients, the mild dehydration of the test conditions proved insufficient to reduce urine flow rates to less than 75 ml. per 60 min. Despite the small number of cases, the data are sufficient to indicate the trend at this stage of the disease. The discrepancy between urea clearance and concentrating ability was even more marked in this group than in group IV, and the urea clearance mean was 28% lower than the concentrating ability. The standard deviation was much less than in previous groups, and the individual results, i.e. for each of the three one-hour periods showed that the range of variation in any one subject was much narrower (observed values all fell within $\pm 2\%$ of the mean). Subjects in group V appeared to have reached a "fixed" state of renal function with respect to both tests. It should be pointed out that at this stage of the disease, renal function tests were no longer of diagnostic interest and were carried out mainly to assess and compare the extent of renal dysfunction by both methods.

One of the subjects in group V (subject 1) had been studied previously one year before, and so it was possible to assess in the same subject the changes in both concentrating ability and urea clearance accompanying deterioration in renal function. The results are shown in Table XV. At the first investigation, this subject was classified in group III (subject 12) and there was a good agreement between the two assessments of renal function.

One year later, the subject was classified in group V (subject 1) and there was a marked discrepancy between the two results.

Correlation between urea clearance and concentrating ability.

~~The results obtained with the various means of concentration~~
Correlation between urea clearance and concentrating ability. showed that in the early stages of chronic renal disease, concentrating ability and urea clearance were very similar, but with further deterioration of renal function (groups IV and V) there was a marked difference between the results. It was therefore of considerable importance to establish if there was any correlation between urea clearance and concentrating ability for the five groups as a whole, despite the difference in values for groups IV and V.

The relationship between the concentrating ability and the standard urea clearance for all 46 subjects (groups I to V) is shown in Fig. 17. The equation to represent the best straight line through this set of data was:

$$C = 0.53 U + 37.4 \dots\dots\dots (8)$$

where C is the concentrating ability and U is the urea clearance, both values being expressed as a percentage of the average normal. The correlation coefficient (r) was 0.88, and was significant at the 0.1% level (P = 0.001). This showed that although the absolute values for the tests differed in certain groups, there was a significant correlation between the tests over the entire range of values.

TABLE XIV.

GROUP V - CONCENTRATING ABILITY AND UREA CLEARANCE IN
RENAL DISEASE PATIENTS WITH URAEMIA.

<u>Subject</u>	<u>Blood urea</u> <u>mg./100 ml.</u>	<u>Concentrating</u> <u>ability %</u>	<u>Urea clearance</u> <u>%</u>
(Mean of 3 estimations)			
1	114	42	15
2	170	41	8
3	141	39	13
4	178	33	6
5	102	44	17
		<u>Mean</u> : 40%	<u>Mean</u> : 12%
		<u>Range</u> : 33 - 44%	<u>Range</u> : 6 - 17%
		<u>S.D.</u> : ± 4	<u>S.D.</u> : ± 5

TABII XV.

DETERIORATION IN CONCENTRATING ABILITY AND UREA CLEARANCE

IN THE SAME SUBJECT.

<u>Subject</u>	<u>Blood urea</u> mg./100 ml.	<u>Concentrating ability</u>			<u>Urea clearance</u>		
		1	2	3	1	2	3
A	40	87%	76%	77%	87%	81%	68%
A (1 year later)	114	43%	42%	40%	16%	16%	14%

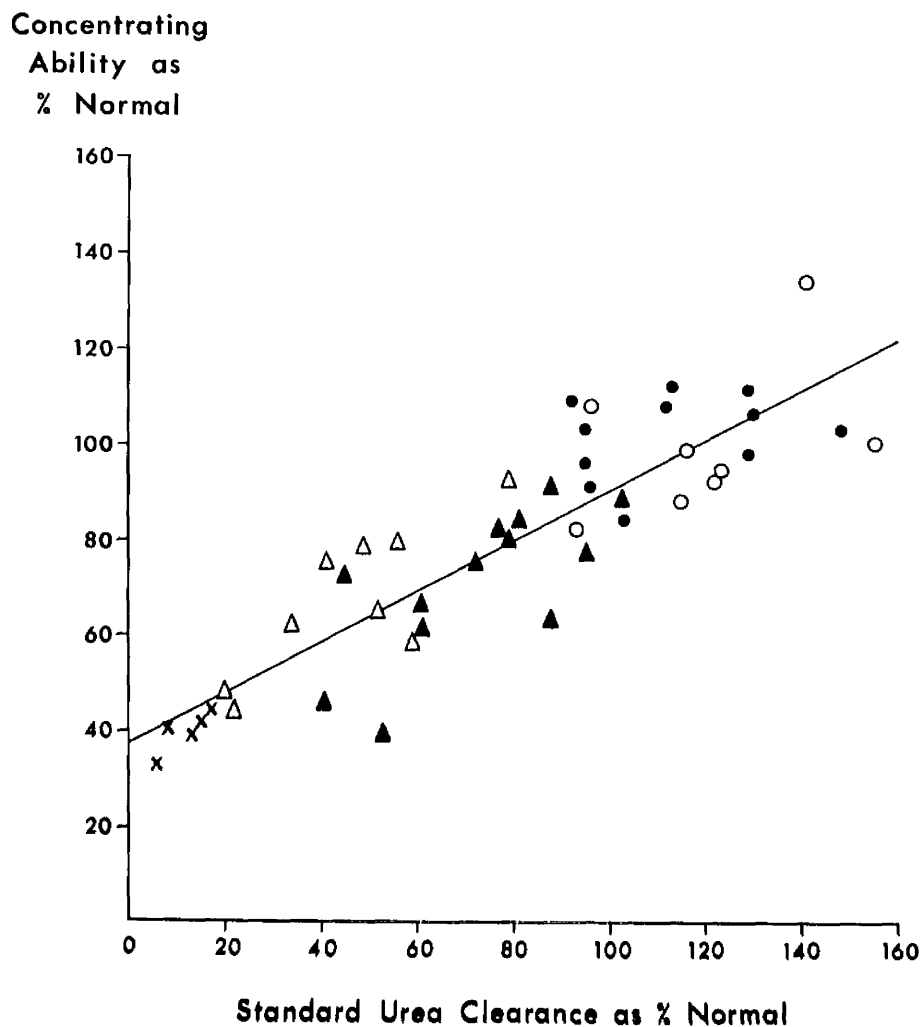


FIGURE 17. RELATIONSHIP BETWEEN UREA CLEARANCE AND CONCENTRATING ABILITY IN CHRONIC RENAL DISEASE.

● Group I ○ Group II ▲ Group III △ Group IV x Group V

POLYURIA IN CHRONIC RENAL DISEASE.

Under the mild dehydration conditions of the concentrating ability test, many of the chronic renal disease patients were unable to achieve urine flow rates less than 75 ml. per 60 min. The occurrence of this polyuria limited the application of the concentrating ability test to such patients.

In normal subjects at urine flow rates greater than 75 ml. per 60 min. it was found that the variation in solute excretion was due to water diuresis. When a diuresis was induced by urea, or Furosemide diuretic, this variation in solute excretion did not occur, and the linear relationship between solute excretion and urine flow rate persisted, even at flow rates greater than 75 ml. per 60 min.

In the chronic renal disease patients the polyuria ranged from 75 - 140 ml. per 60 min.; this represented only a mild diuresis. All patients were on fluid restriction for the 1 - 2 hour period prior to the test, and were under mild dehydration during the test. It seemed unlikely therefore that this polyuria could be caused by a water diuresis.

Solute excretion rates were investigated in subjects with renal polyuria, to assess the variation which occurred at urine flow rates greater than 75 ml. per 60 min. These values were then compared with solute excretion rates at urine flow rates less than 75 ml. per 60 min., in a group of similar chronic renal disease subjects. This comparison could be carried out only on subjects with approximately the same state of renal function, since patients with widely differing renal function would obviously have different solute excretion rates at a given urine flow rate.

The relationship between solute excretion and urine flow rate for both the polyuria group and the control group are shown in Fig. 18. The urea clearance values in these subjects ranged from 10 - 30% of the average normal.

The linear relationship between solute excretion and urine flow rate persisted under conditions of a polyuria due to renal disease. In this respect, the polyuria of renal disease was similar to osmotic diuresis in normal subjects, and unlike a water diuresis in normals.

Urine osmolality in uraemia patients.

One of the most characteristic features of advanced renal disease is the inability to vary the composition of the urine under differing conditions of fluid intake, the so-called "fixed" specific gravity and "fixed" osmolality urine. It has been shown in the present study that in normals at urine flow rates less than 75 ml. per 60 min., the urine osmolality was relatively constant and predictable, ranging from a minimal average value of 809 mOsm. per Kg. water at urine flow rate 75 ml. per 60 min., to a maximum average value of 1200 mOsm. per Kg. water at 14 ml. per 60 min. The transition to urine flow rates greater than 75 ml. per 60 min. was accompanied by a marked variation in urine osmolality which was no longer predictable.

Urine osmolality in uraemia patients was compared at urine flow rates below and above 75 ml. per 60 min. The results for the uraemia patients are compared with those in normal subjects over the same range of urine flow rates, and are shown in Fig. 19. For the uraemia patients, there was no significant change in urine osmolality at urine flow rates above 75 ml. per 60 min. The range of variation in these subjects was small and the mean

SOLUTE EXCRETION
mOsm. 60min.

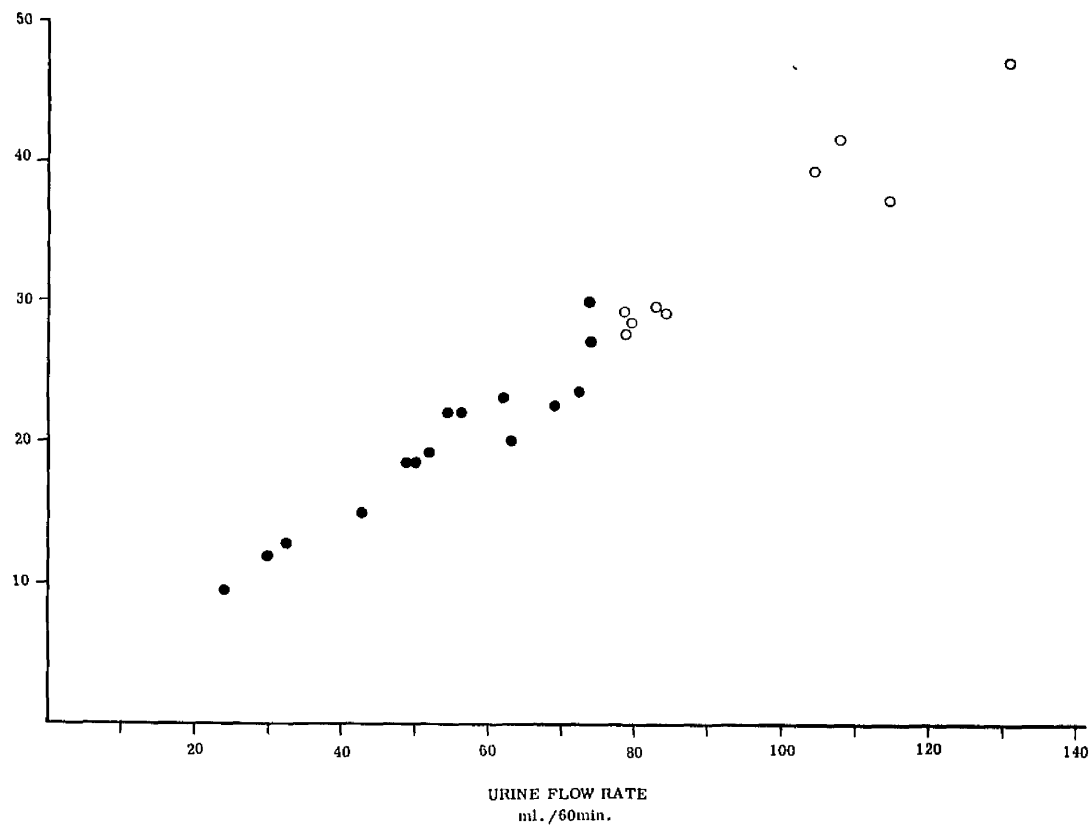


FIGURE 18. SOLUTE EXCRETION RATES IN CHRONIC RENAL DISEASE PATIENTS.

- Subjects with urine flow rate less than 75 ml. per 60 min.
- Subjects with renal polyuria.

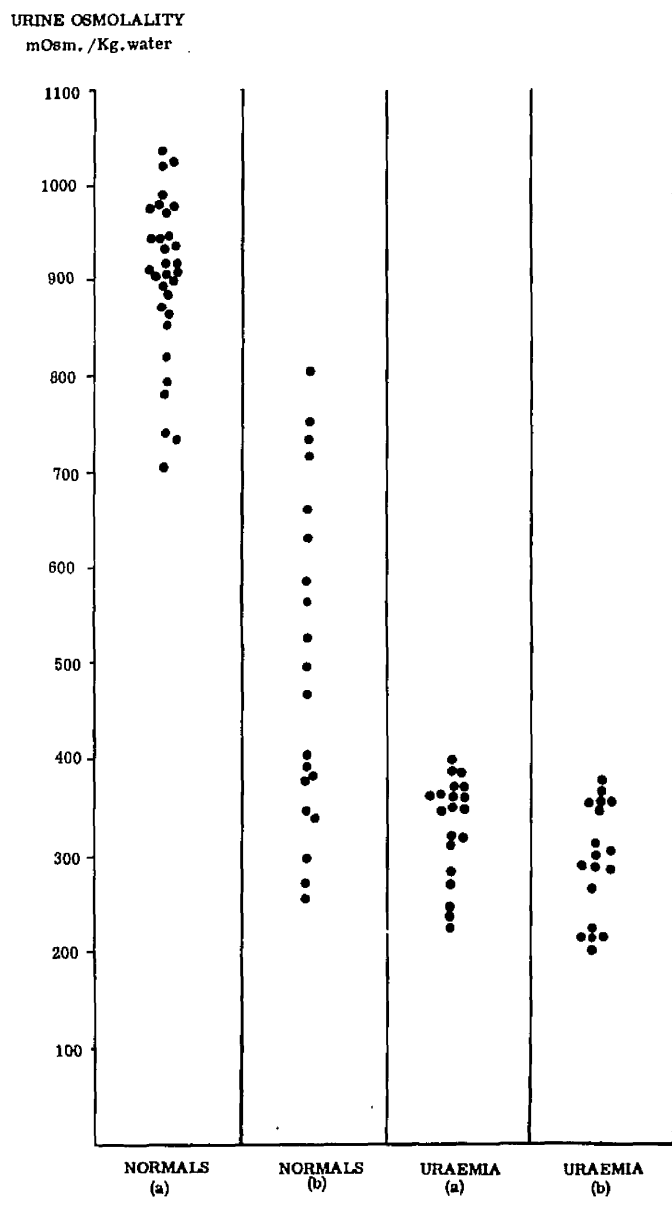


FIGURE 19. URINE OSMOLALITY IN URAEMIA PATIENTS COMPARED WITH NORMAL SUBJECTS.

- (a) Urine flow rate 75 ml. per 60 min.
- (b) Urine flow rate 75 - 140 ml. per 60 min.

values indicated that urine osmolality was "fixed" in the region of the normal serum osmolality.

Serum osmolality in chronic renal disease.

Serum osmolality in chronic renal disease. For all practical purposes a constant (Hendry, 1961, and Olmstead and Roth, 1957). The normal range used as a reference in the present study was 281 - 297 mOsm. per Kg. water (Mean 289, S.D. ± 4).

The concentrating ability test is based on the evidence that within the limits 14 - 75 ml. per 60 min., variation in urine osmolality is a function of the urine flow rate. Within these narrow limits serum osmolality should remain unchanged and would therefore not affect the concentrating ability test. For this reason, the concentrating ability was based only on urine osmolality, and serum osmolality was not taken into account.

The only situation in which serum osmolality might be expected to affect concentrating ability results would be if the subject had initially a grossly abnormal serum osmolality. This might apply to patients with raised blood urea concentration in whom one would expect to find an increase in serum osmolality. Serum osmolality was measured in subjects with (a) moderately raised blood urea concentration, (b) uraemia, and the results are shown in Table XVI.

In the group with moderately raised blood urea, only 2 of the 10 subjects had a serum osmolality outside the normal range. In the uraemia group, the serum osmolalities were all outside the normal range. The mean increase in serum osmolality for the uraemia patients was 7%, and the

maximum was 10% (serum osmolality 318). In comparison to the increase in blood urea, changes in serum osmolality were small, and would not introduce any serious error into the assessment of renal function based only on urine osmolality.

TABLE XVI.

SERUM OSMOLALITY IN CHRONIC RENAL DISEASE.

(a) Patients with moderately raised blood urea.

(b) Uraemia patients.

(a)	<u>Subject</u>	<u>Blood urea</u> mg. per 100 ml.	<u>Serum osmolality</u> (mOsm./Kg. water)
	1	43	292
	2	89	294
	3	79	306
	4	46	290 Mean : 296
	5	45	296
	6	53	295
	7	86	315
	8	53	293
	9	67	289
	10	55	285
<hr/>			
(b)	1	180	314
	2	114	301
	3	101	301 Mean : 310
	4	186	315
	5	141	318

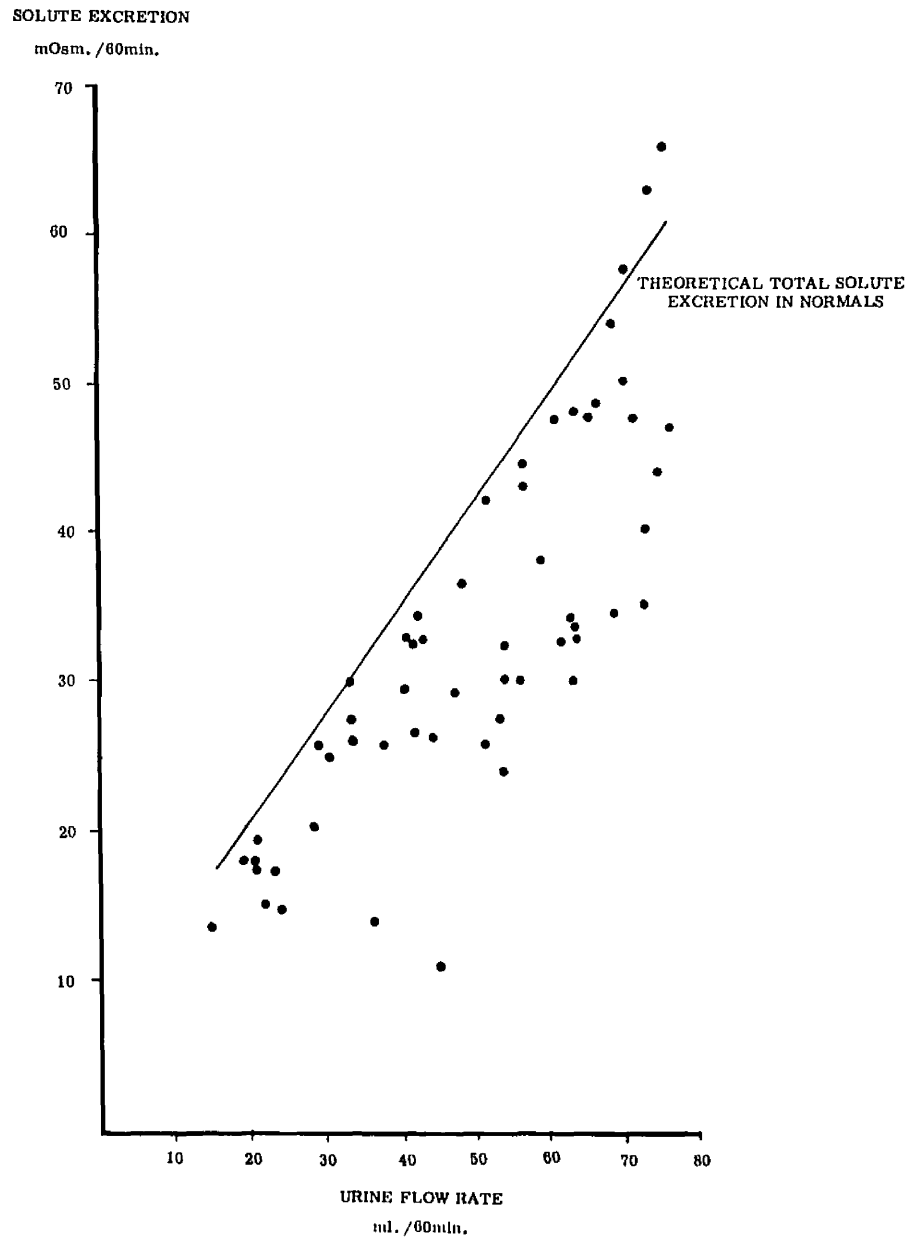
APPLICATION OF THE CONCENTRATING ABILITY TEST TO CONDITIONS OTHER THAN CHRONIC RENAL DISEASE.

In applying the test to chronic renal disease patients, an abnormally low urine osmolality and hence a low concentrating ability was taken as an index of renal dysfunction. The conditions of the test were designed so that no extraneous factors e.g. high fluid intake, low temperature etc., would cause falsely low concentrating ability. It was possible, however, that other abnormal clinical conditions apart from chronic renal disease might be associated with an abnormally low concentrating ability.

(1) Concentrating ability in patients with angioneurotic oedema.

Patients in this category suffered from periodic oedema, usually of abrupt onset. The extent of the oedema varied from subjects with only mild facial oedema, to subjects with generalised peripheral oedema. There was no evidence of any organic lesion to account for the occurrence of oedema. Subjects in this group had normal serum proteins and cholesterol; blood urea and urine findings were normal. None of the subjects had any previous history of renal disease. The onset of the oedema was, however, invariably associated with some situation of emotional stress in the life of the subject.

The group consisted of 23 patients, and a total of 58 specimens were obtained from this group. Oedema patients were under the same conditions as the renal disease groups, prior to and during the test. The relationship between solute excretion and urine flow rate for this group is shown in Fig. 20, for urine flow rates less than 75 ml. per 60 min. The line shown in Fig. 20 represents the theoretical relationship between solute excretion and urine flow rate for normal subjects (equation (2) p. 57).



**FIGURE 20. RELATIONSHIP BETWEEN SOLUTE EXCRETION RATE AND URINE FLOW RATE
IN ANGIONEUROTIC OEDEMA PATIENTS COMPARED WITH NORMALS.**

● Oedema patients

In this group, the distribution of solute excretion rate was very different from that in normal subjects. At any given urine flow rate, the mean solute excretion for the oedema group was considerably less than in normals, and consequently, the concentrating ability in this group was less than normal. The scatter of results indicated that there was a much wider variation compared with normal subjects (cf. Fig. 6). This increased scatter also occurred in the results from any one subject in this group, and both solute excretion and urine flow rate were subject to greater variation than in any of the previous groups.

Allowing for normal variation about the line shown in Fig. 20, some of the results obtained from oedema patients lay within the normal range. There were, however, a considerable number of points which lay without the normal range. From the results obtained on normal subjects, and control patients, the minimal normal concentrating ability recorded was about 80%. In this group of angioneurotic oedema patients, only concentrating ability less than this was considered abnormal. By this classification, 5 of the subjects had considerably abnormal concentrating ability. Urea clearance tests were carried out on these subjects to assess renal function, and the results for both concentrating ability and urea clearance are shown in Table XVII. Despite the consistently low concentrating ability, urea clearance values all lay within the accepted normal range. Coupled with normal urine findings, and no other evidence of renal disease, the results suggested that renal function in these subjects was normal.

The major contributors to urine osmolality are urea and electrolyte which collectively account for 90% or more of the total urine osmolality

TABLE XVII.

UREA CLEARANCE IN SUBJECTS WITH ABNORMALLY LOW CONCENTRATING ABILITY.

<u>Subject</u>	<u>Concentrating ability %</u>	<u>Urea clearance %</u>
(Mean of 3 estimations)		
1	64	81
2	63	81
3	61	77
4	64	83
5	66	86

in normal subjects. The normal urea clearance values obtained for the 5 subjects implied that urea excretion was normal in these subjects. Consequently, the lowered solute excretion and lowered concentrating ability in these subjects must be due to a reduction in the electrolyte excretion.

Electrolyte excretion was measured by conductivity in subjects with abnormally low concentrating ability, and the results are shown in Fig. 21. The line represents the theoretical relationship between electrolyte excretion and urine flow rates in normals, obtained using equation (7), p. 81. The results showed that electrolyte excretion was considerably sub-normal in these subjects, thus accounting for the low concentrating ability.

(ii) Clinical conditions associated with abnormal fluid intake.

Renal concentrating ability was investigated in a patient with a history of compulsive water drinking, of more than a year's duration. The patient had no known history of renal disease and no symptoms suggestive of renal disease at the time of investigation. The results are shown in Table XVIII. Urine osmolality was low, concentrating ability was sub-normal, but the urea clearance was within normal limits.

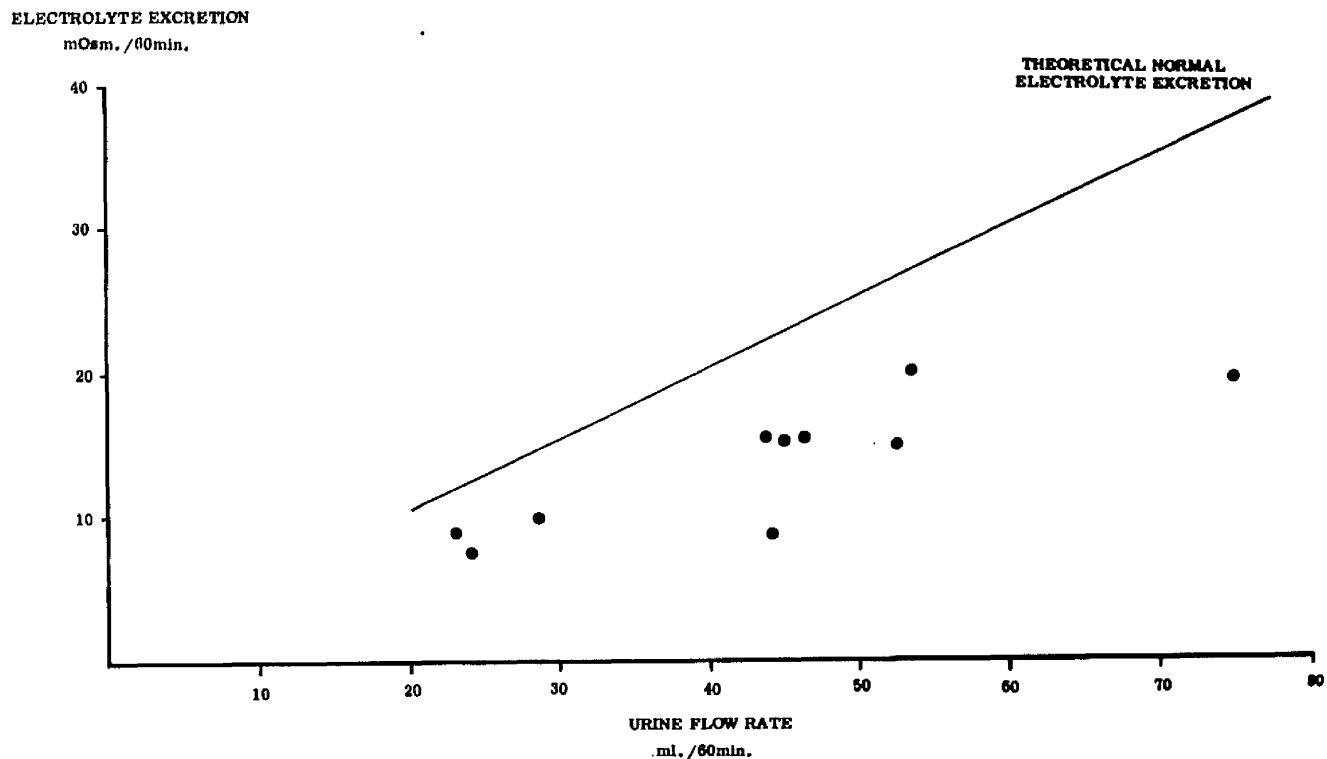


FIGURE 21. ELECTROLYTE EXCRETION IN ANGIONEUROTIC OEDEMA PATIENTS WITH ABNORMALLY LOW CONCENTRATING ABILITY COMPARED WITH NORMALS.

TABLE XVIII.

COMPARISON OF CONCENTRATING ABILITY AND UREA CLEARANCE.

in (a) A subject with compulsive water drinking.

(b) A subject with anorexia nervosa.

<u>Urine osmolality</u> (mOsm./Kg. water)		<u>Serum osmolality</u> (mOsm./Kg. water)	<u>Concentrating ability</u> %
(a)	433	290	49
	519		57
(b)	186	297	19
	153		18

<u>Urine urea</u> (g./l.)		<u>Blood urea</u> (mg. per 100 ml.)	<u>Urea clearance</u> %
(a)	12.6	21	91
	14.7		101
(b)	4.8	16	39
	4.0		44

(iii) Deficient dietary intake.

Urea clearance and concentrating ability were studied in a patient suffering from anorexia nervosa. In this condition, there is usually some underlying source of worry or emotional disturbance which manifests itself outwardly in a loss of the desire to eat. The presentation of food to such subjects invariably produces nausea. The results are shown in Table XVIII. Over a period of almost a year, the subject had a generalised dietary deficiency, and this was associated with both sub-normal concentrating ability and urea clearance. The subject had no previous history of renal disease, and no symptoms suggestive of renal disease at the time of investigation.

DISCUSSION.

OSMOTIC PRESSURE.

The osmotic pressure of urine varies considerably in normal subjects, and this variation is effected largely by the kidney in response to the needs of the body for conservation of water and solutes. One of the major functions of the kidney is the production of a concentrated urine, and in this complex process, osmotic gradients are established within the kidney to allow the final concentration of the urine. Under conditions of anti-diuresis, urinary osmotic pressure, in as much as it reflects the concentrating capacity of the kidney, should bear some relationship to the functional state of the kidney.

In the assessment of renal concentrating ability, osmotic pressure has many advantages over methods based on specific gravity. Osmotic pressure varies with the number of particles present in solution, irrespective of their size. As a measure of total solute concentration, it has more physiological significance than specific gravity, since body fluids respond to changes in osmotic pressure, not to changes in specific gravity.

METHODOLOGY.

One of the essential features of any biochemical test for routine use is that it should be based on a reliable and reproducible method. Freezing point osmometry represents such a technique and is ideal for the routine estimation of osmolality in body fluids. Osmolality can be measured rapidly and accurately using the Fiske osmometer, and the error involved is much less than in many other methods used routinely.

Osmotic pressure varies with temperature, and one criticism which has been made about freezing point osmometry is that the measurement is made at

0° on body fluids which have a normal environmental temperature of 37°. For this reason, the use of methods based on vapour pressure has been suggested, since this would permit the measurement of osmotic pressure at 37°. A comparison of results at 0° using the Fiske osmometer and at 37° using a vapour pressure method was carried out by Steinmetz and Ludlum (1964), using plasma, urine and synthetic solutions containing urea, sodium and chloride.

With the synthetic solutions, a good correlation was obtained between the two methods, over the entire physiological range, showing that there was no significant change in the osmotic activity of the major urinary solutes at the two different temperatures. With urine samples obtained from both normals and chronic renal disease patients, the correlation between the methods was good for dilute and moderately concentrated urine. With highly concentrated urine, there was an increased scatter of results, with no consistent trend. This variation in highly concentrated specimens was considered to reflect a change in the osmotic activity of some of the less predominant urinary solutes, e.g. phosphates and urates.

The technical advantages of freezing point osmometry make it a more suitable method for routine use than methods based on vapour pressure measurement. Steinmetz and Ludlum concluded that the overall agreement between the two methods was sufficiently good to permit the use of freezing point osmometry in most physiological and pathological conditions.

When working with highly concentrated urine specimens, the solubility product of certain of the constituents may be exceeded during the cooling procedure. This applies particularly to urine samples at concentrations

greater than about 750 mOsm. per Kg. water. In urine, this precipitate consists mainly of uric acid or urates, which no longer contribute to urine osmolality. The error introduced by the removal of this uric acid is small, however, and precipitation of the entire urinary uric acid would represent an error of only 3 - 4 mOsm. per Kg. water (Hendry, Harrison and Fletcher, 1964). This difficulty can be overcome by diluting highly concentrated specimens before measuring osmolality, since it is unlikely that precipitation will occur in the diluted sample. The osmolality of the original sample can then be found using the dilution factor F described on p. 38.

EXPERIMENTAL CONDITIONS.

Reliable results based on urine collection depend almost entirely on the cooperation of the subjects, and their ability to void urine specimens at regular intervals. In the present study, non-catheter specimens were used throughout. In practice, the majority of normal and renal disease subjects encountered were able to comply with the test conditions, and only a few subjects had to be excluded, e.g. patients with bladder and/or prostatic abnormalities.

Specimens from patients were collected at hourly intervals, since this usually represented a suitable period for voiding specimens and also provided an adequate volume for collection and measurement when patients were under mild dehydration or normal hydration. Under conditions of diuresis, shorter collection periods of 15, 20, or 30 minutes were used.

The state of hydration is one of the major factors which affects urine concentration (de Wardener and Herrheimer, 1957; Habener et al. 1964). When an assessment of renal concentrating ability was carried out, fluid intake was

controlled both prior to and during the test period. The relationship between fluid intake and the state of hydration was a very difficult one to gauge. It was dependent on factors such as the rate of water absorption, insensible water loss via the lungs and skin, and production of water of metabolism, all of which vary in different individuals.

An attempt was made to eliminate variation due to environmental factors by carrying out all tests on patients in a room which was maintained at a temperature of 20 - 22° during the test. The state of activity of the patients was roughly similar, in that they remained seated during the test period, usually reading. Patients were allowed to smoke, since the anti-diuretic effect was in keeping with the low urine flow rates required by the test.

Dietary factors affect renal function, in particular the dietary content of protein and of salt affects the urine concentrating process, and maximal concentrating ability is achieved on a diet which has a high protein and a low salt content (Meroney et al., 1958). Urea clearance is also affected by the protein content of the diet (Nelson and Bang, 1948; Kleeman et al., 1965). It is therefore essential in any study of renal function that all subjects have a reasonably standard intake of these dietary items. This is an important consideration in chronic renal disease subjects, many of whom may be on a prescribed low-protein diet.

Urine osmolality following dehydration as an index of renal function.

Most tests of renal concentrating ability based on osmolality measurement have used either urine osmolality or the urine:plasma osmolality ratio following a period of dehydration. There is considerable variation in the

literature regarding the optimal dehydration period for such a test, and in the range of normal values obtained.

Most groups favoured a 12 - 14 hour dehydration period since this could conveniently be carried out overnight (Frank et al., 1957; De Leon et al., 1960; Jacobson et al., 1962). Jones and de Wardener (1956) used a 48-hour period, and Dashe et al., (1963) used a $6\frac{1}{2}$ -hour period. In the present study, a 14 - 15 hour dehydration period was used.

The normal range obtained was 585 - 1104 mOsm. per Kg. water and when this was repeated on the same subjects after an interval of 14 days, a range of 713 - 1206 mOsm. per Kg. water was obtained (Table III, p. 54). For the group as a whole, the results were reproducible, although in some individuals the variation was considerable. The most disturbing feature of the results was the variation within the group, representing a very wide range in normal subjects under standard conditions.

A wide normal range was apparent in most similar experiments in the literature. De Leon et al., (1960) and Dashe et al., (1963) obtained similar ranges of 756 - 1496 and 741 - 1410 mOsm. per Kg. water respectively, despite the much shorter dehydration period of the latter group. Jacobson et al., (1962) obtained a range of 855 - 1335 mOsm. per Kg. water.

Considerable individual variation was also apparent, and Jacobson et al. (1962) noted that at the 13th hour, 5 subjects had a urine concentration below the lower limit of normal. Conversely, in 6 other subjects, urine concentration was greater at the 13th hour than at the 14th hour. Black (1963) pointed out that the most concentrated urine specimen was not necessarily at the end of the dehydration period, but the early morning specimen (circa

4 - 5 a.m.), coinciding with the trough of the diurnal excretory rhythm.

The only reasonably concise index of renal concentrating ability was that of Jones and de Wardener (1956) ranging from 1009 - 1301 mOsm. per Kg. water. The practical difficulties involved in a 48-hour dehydration period are, however, obvious and would make such a test totally unsuitable for routine investigation.

The response to dehydration in normals was very variable. It was concluded from the present series and the results reported in the literature that no precise index of concentrating ability could be obtained by measuring urine osmolality following dehydration.

Urine osmolality in relation to urine flow rate as an index of renal concentrating ability.

Further studies on the same normal subjects showed that many of the subjects could achieve a higher osmolality throughout the day (under normal hydration or only mild dehydration) than after a prolonged dehydration period (Table IV, p. 55). Jones and de Wardener (1956) made a similar observation in their series and noted that some subjects on control days (i.e. under normal hydration) could attain a urine osmolality similar to that following a 48-hour dehydration period. These results proved that long periods of dehydration were not essential to produce a concentrated, high osmolality urine.

As a result of this finding, changes in osmolality, solute excretion rate and urine flow rate were studied in normal subjects under various states of hydration, to establish the conditions under which a highly concentrated urine could normally be produced. When solute excretion rate was plotted against

urine flow rate, it was found that the data fell into two distinct groups (Fig. 5, p. 56). In the urine flow range 14 - 75 ml. per 60 min., a linear relationship existed between urine flow rate and solute excretion rate, while at flow rates greater than this there was an apparently random scatter of results.

One significant feature of this relationship was the high degree of correlation between the two parameters ($r = 0.94$), suggesting some regularity in the underlying physiological mechanism. In antidiuresis, the state of the kidney has been likened to a mild but variable state of osmotic diuresis (Baldwin et al., 1955). This suggests that the linear increase in solute excretion rate with urine flow rate over the range 14 - 75 ml. per 60 min. may well represent an "endogenous" osmotic diuresis occurring in the anti-diuretic state.

The minimal observed urine flow rate of 14 ml. per 60 min. corresponded to a theoretical osmolality of 1200 mOsm. per Kg. water and the maximal urine flow rate of 75 ml. per 60 min. to a theoretical osmolality of 809 mOsm. per Kg. water. This range of values was similar to that attained after prolonged dehydration, despite the very different conditions of the present test. This variation in urine osmolality over a narrow range of urine flow rates and the more random variation at higher urine flow rates showed that little reliance could be placed on urine osmolality as an index of renal concentrating ability, unless the urine flow rate was known. It seemed probable that the wide range of osmolalities attained following dehydration was related to differences in urine flow rate.

By means of this relationship between urine flow rate and solute output

in the range 14 - 75 ml. per 60 min., it was possible to derive the theoretical osmolality corresponding to any urine flow rate within the range. The observed urine osmolality could then be compared with the theoretical value, and the ratio observed: theoretical osmolality would give a measure of renal concentrating ability.

In normal subjects at urine flow rates over 75 ml. per 60 min. it was possible to discern a hyperbolic relationship between urine flow rate and urine osmolality (Fig. 7, p. 61) but the scatter of results was too great to obtain any precise index of normal renal function from this data. Most renal disease patients have some limited ability to produce a slightly hypertonic urine, and only in regions of extreme concentration would they exhibit a concentrating defect. At flow rates greater than 75 ml. per 60 min., normal subjects produce urine which is only slightly hypertonic, and at such urine flow rates, urine osmolality would have little practical value as an index of renal function.

It has been shown that in chronic renal disease, concentrating ability is impaired prior to diluting ability (Kleeman, Adams and Maxwell, 1961). In addition, more demands are normally made on the concentrating ability of the kidney than on the diluting ability, when normal amounts of solute and water are being handled. For both reasons, urine osmolality at low urine flow rates (i.e. less than 75 ml. per 60 min.) is the most effective measure of renal function.

It was, however, important to establish the reasons for the increased scatter of normal data at higher urine flow rates, in order to define more clearly the limits within which the relationship between solute excretion

and urine flow rate would hold. To eliminate one possible source of variation, patterns of solute and water excretion were studied during the course of a water diuresis, in a series of individual subjects, rather than as a group.

Water diuresis in normal subjects.

During the course of a brisk water diuresis, the urine flow rate increased sharply to a maximum and then returned to pre-diuresis values (Fig. 9, p. 68). The transition from steady urine flow rates to rapidly increasing urine flow rates occurred abruptly in the region of 75 ml. per 60 min., suggesting that this region might be associated with the cessation of antidiuretic hormone activity. In support of this, Thomas (1964) studying solute excretion in normals showed that when vasopressin was administered during a sustained water diuresis, there was an abrupt fall in urine flow rate (10 - 15 min. after vasopressin). Before administration of vasopressin, urine flow rates ranged from 10 - 15 ml. per min. and after vasopressin, urine flow rates ranged from 0.27 - 1.1 ml. per min. (16.2 - 66 ml. per 60 min.), suggesting that a range of urine flow rates 14 - 75 ml. per 60 min. would be compatible with antidiuretic hormone activity.

In all 4 subjects studied in this work, the solute excretion pattern differed from that of the urine flow rate, and the peak solute excretion occurred 30 - 45 min. before the maximal urine flow rate following an oral load of 500 ml. water. This increase in solute excretion was apparent in total solute excretion, urea excretion, and to a minor extent in chloride excretion. After reaching the maximal value, solute excretion decreased while the urine flow rate was still rising. In the post-diuresis period,

the solute excretion rate still continued to fall, and was much less than in the pre-diuresis period.

Haas, Holdaway and Robinson (1965) obtained similar results studying only urea excretion in normals during a brisk water diuresis. In their experiments, following an oral load of one litre of water, the peak urea excretion occurred $1 - 1\frac{1}{2}$ hour prior to the water diuresis peak. They also observed low urea excretion values in the post-diuresis period. Similar results in urea excretion were obtained when the diuresis was induced using saline, but when an osmotic diuresis was induced using urea, the urea excretion rose consistently with the urine flow rate.

The increase in urea excretion in the present study and that observed by Haas et al., (1965) is consistent with the current theories relating to urea storage within the kidney. It is now generally accepted that an intrarenal store of urea exists during antidiuresis (Berliner et al., 1958; Schmidt-Nielsen, 1958), and the increase in urea excretion may represent a 'wash-out' effect by which this urea is removed.

The present observations showed that in addition to an increase in urea, considerable changes in the excretion of other solutes also occurred. The marked decrease in solute excretion in the immediate post-diuresis period may have some significance in relation to the counter current theory. It seems likely that this may represent a re-accumulation of the ions necessary to regenerate the concentration gradient within the inner medulla. Similarly the increase in solute excretion at the onset of the diuresis may represent a removal of the ions accumulated in the concentration gradient during anti-diuresis. Haas et al., (1965) considered that the reduction in urea excretion

in the post-diuresis period also represented a re-accumulation process, and they were able to show that the increase in urea excretion in the pre-diuresis period, and the decrease in the post-diuresis period were roughly of the same order.

During water diuresis, the patterns of water and solute excretion were "out of phase". One consequence of this was that at any given urine flow rate, the solute excretion rate could vary depending on whether the solute excretion pattern was rising or falling. This variation in solute excretion, during a water diuresis probably accounted for much of the scatter previously observed in normal subjects at high urine flow rates (Fig. 5, group B, p. 56).

After urine is formed by the kidney, it must pass through the renal pelvis, the ureter and the bladder, i.e. the renal tract 'dead-space'. The transient increase in solute excretion observed at the beginning of a water diuresis could represent an increase due to mixture of concentrated urine contained in the renal tract "dead-space" with more dilute urine. This would apply particularly when a water diuresis occurred after a period of prolonged antidiuresis. Haas et al., (1965) calculated that the increase in urea excretion at the onset of a water diuresis was too great to be contributed by concentrated urine contained in the renal tract "dead-space."

In the present study, the effect of successive periods of water diuresis was studied, with only very brief periods of antidiuresis between the diuresis. A discrete increase in solute excretion occurred with each diuresis, and there was no evidence of a reduction in this increase of solute excretion with successive diuresis. This implied that the increase represented a removal of solute from the kidney, rather than from the renal tract "dead-space."

Diuresis with increased solute excretion in normal subjects.

(i) Urea induced diuresis. (Fig. 11, p. 74)

In contrast to a water diuresis, under conditions of an osmotic diuresis induced by urea, the patterns of solute and water excretion were "in phase," throughout the diuresis. Consequently, when solute excretion rates were plotted against urine flow rates, the linear relationship persisted, even at flow rates greater than 75 ml. per 60 min.

(ii) Furosemide induced diuresis. (Fig. 13, p. 77)

The diuretic Furosemide acts by blocking the tubular reabsorption of sodium and chloride, and the net effect is similar to an osmotic diuresis with sodium and chloride as the loading solutes. With this diuretic the patterns of solute and water excretion were also "in phase" throughout the diuresis. As in the case of urea, the linear relationship between solute excretion and urine flow rate continued at flow rates greater than 75 ml. per 60 min.

One interesting feature of this diuresis was the substantial increase in urea excretion which occurred. Haas et al., (1965) had a similar finding with a saline induced diuresis. The increase in urea excretion did not appear to be restricted to conditions of a water diuresis, but occurred during the Furosemide diuresis, when the output of other solutes was also increasing.

In relation to the concentrating ability test, the results showed that the linear relationship between solute excretion and urine flow rate was not restricted to 14 - 75 ml. per 60 min. under conditions of

a diuresis induced by urea or Frusemide. Under these conditions, the solute excretion rate continued to rise linearly with the urine flow rate, even at flow rates greater than 75 ml. per 60 min.

Renal concentrating ability in subjects with chronic renal disease.

The reliability of the test based on concentrating ability as an index of renal function could be assessed only by comparison with one of the generally accepted tests of renal function. The test chosen for comparison was the Van Slyke urea clearance test, since it gives a measure of general renal function, and is suitable for application on a routine basis.

Despite the basic differences between the tests, they had certain features in common in that the conditions required by the concentrating ability test were suitable for urea clearance, the periods of urine collection were identical, and it was therefore possible to carry out both tests simultaneously. Both concentrating ability and urea clearance results were expressed as a percentage of the average normal, and consequently it was valid to compare directly the two assessments of renal function.

To assess its reproducibility, the test was applied first to a group of normal controls, and then to a group of control patients (Table VIII, p. 86; Table IX, p. 88). The concentrating ability test yielded reproducible results with a mean value close to 100% for both groups; the range of variation of individual results over the three collection periods was also narrow. The results in control patients confirmed that the hospital regime in no way altered the concentrating ability of normal subjects, nor did the recumbent state prior to the test affect the results.

For both groups, the urea clearance mean was considerably higher than

100%, and most of the individual values fell into the upper part of the normal range quoted by Peters and Van Slyke (1932). One factor which is known to have a considerable effect on urea clearance is dietary protein intake (Murdaugh and Schmidt-Nielsen, 1957; Kleeman et al., 1965). Diet histories were not investigated in this study, but it does seem probable that the present subjects had a habitually higher intake of protein than the original group used by Van Slyke. Other minor factors which differed in the present study were the method of estimation of urea and the age range of the control subjects which was on the whole younger than those used in the original data of Peters and Van Slyke (1932).

Patients with chronic renal disease were sub-divided into groups on the basis of their blood urea concentrations. Clinically, the most interesting group for the application of the test was Group III, i.e. patients with normal blood urea concentration (Table XI, p. 91). In this group, there was a close agreement between both assessments of renal function with respect to the mean values and the normal ranges. In general, the concentrating ability test appeared to be equally effective in the detection of most of the commonly encountered forms of chronic renal disease, and did not appear to be restricted to any specific disease types.

Some subjects in group III exhibited both normal concentrating ability and urea clearance, despite symptoms, and/or signs consistent with chronic renal disease. This anomaly was also observed by Jacobson et al., (1962) who noted several instances of renal disease subjects with urine osmolality greater than 800 mOsm. per Kg. water, and a few greater than 1000 mOsm. per Kg. water. These results indicated that the presence of a highly concentrated

urine did not always preclude the possibility of renal disease.

In subjects with moderately raised blood urea concentrations (group IV, Table XIII, p. 96) the tests no longer gave comparable results. In all cases, the urea clearance was lower than the concentrating ability, and for the group as a whole, the urea clearance mean was 20% lower than the concentrating ability. This was in contrast to the normal and patient control groups I and II in which the urea clearance was 10 - 20% higher than the concentrating ability.

In uraemic patients (group V, Table XIV, p. 99), this discrepancy was further emphasised with a 30% difference between the mean values for the two tests. The number of patients studied in this group was restricted by the wide occurrence of polyuria in this stage of chronic renal disease. The variation of results in this group was small, however, and the data gave some indication of the trend at this stage of the disease, despite the small number of cases. Subjects in this category appeared to have reached a fixed state of renal function with respect to both tests.

Urea clearance compared with concentrating ability in the assessment of renal function.

In attempting to account for the observed discrepancy between the two tests in advanced renal disease, some of the basic differences between the tests must be taken into consideration. In the estimation of urea clearance, the urinary urea excretion rate is related to blood urea concentration. In chronic renal disease in the advanced stages, urea retention is one of the cardinal features. This results in very low clearance values in subjects with uraemia, irrespective of the absolute concentration of urinary urea.

The concentrating ability test was based only on urine osmolality, and no attempt was made to express the results in the form of osmolal clearances. The justification for this lies in the evidence that in normal subjects, serum osmolality is for all practical purposes a constant (Olmstead and Roth, 1957; Hendry, 1961), and even in uraemic subjects in whom one might expect an increase in serum osmolality, this increase is small. This was confirmed in the present study in which the maximal increase in serum osmolality in a uraemic patient was 10%. In addition, the present test was based on evidence that the major factor in determining urine osmolality was the urine flow rate, and that within the region 14 - 75 ml. per 60 min., urine osmolality was a function of the urine flow rate.

Studies of urea and electrolyte excretion in normal subjects over the range of urine flow rates 14 - 75 ml. per 60 min. showed that the major portion of solute excretion and hence of osmolality was contributed by electrolyte (Fig. 16, p. 82). Consequently, a difference between urea clearance and concentrating ability could reflect a difference in electrolyte excretion. This would suggest that in the more advanced stages of renal disease, the impairment of urea excretion may exceed that of electrolyte excretion. This is consistent with the known ability of the chronically diseased kidney to excrete an increased quantity of salt (Bricker, Mees and Klahr, 1963; Doig, Lawrence and Wright, 1963).

In assessing which of the tests gives a more reliable index of renal function in the advanced stages of renal disease, it should be pointed out that many subjects with urea clearances in the region 5 - 10% of normal can continue to lead reasonably normal lives for several years, provided they do

not succumb to infection. Although such values may represent the true state of urea handling by the subjects, it is possible that this may no longer reflect the functional state of the kidney with respect to other solutes. The concentrating ability for these patients (30 - 40% of normal) seems to be a more realistic index of their residual functional capacity, and more compatible with their general state of activity.

The concentrating ability test appeared to be as sensitive as the urea clearance in the detection of renal impairment in the early stages of renal disease. In the more advanced stages of renal disease there was a marked discrepancy between the two tests. Despite this difference, there was a significant correlation between the tests over the entire range of values (Fig. 17, p. 101).

Advantages and limitations of the concentrating ability test.

The test was found to give more reliable results than methods based on specific gravity or involving long periods of dehydration. Only a minimal amount of fluid deprivation was required which represented a considerable advantage in its practical application to patients. The test allowed a quantitative assessment of renal concentrating ability provided the urine flow rate was known accurately.

If reliable results were to be obtained, however, considerable attention had to be given to the control of fluid intake prior to, and during, the test. Accurately timed and complete urine collections were essential, in view of the low urine flow rate. One of the major limitations was the narrow range of flow rates over which it was applicable. This affected particularly the advanced cases of renal disease in whom polyuria was commonly encountered.

In renal disease subjects with polyuria, it was observed that there was little change in urine osmolality at flow rates above or below 75 ml. per 60 min., in contrast to normal subjects who exhibited a marked change in urine osmolality at flow rates above 75 ml. per 60 min. (Fig. 19, p. 105). One consequence of this was that solute excretion rates for renal disease subjects with polyuria varied linearly with urine flow rate, even at flow rates up to 130 ml. per 60 min. This was analogous to the results obtained in normal subjects when diuresis was induced using urea, or Frusemide, both of which produced an increased solute load.

The mechanism of polyuria in renal disease has never been adequately explained. It was originally attributed to destruction of specific tubular sites, but its occurrence in different pathological conditions suggests that this is unlikely, and implies that some general mechanism must be involved. One explanation is that a reduction in the total number of functional nephrons results in a normal solute load being shared among a smaller number of functional units. Each nephron would then handle a greater than normal solute load, and the situation would be analogous to a continuous osmotic diuresis (Bricker, Dewey, Lubowitz, Stokes and Kirkenasgaard, 1959).

In an attempt to test this hypothesis, Franklin, Neall and Merrill (1959) studied the effects of decreased solute loads in subjects with renal polyuria and impaired concentrating ability - the reduction in filtered load was expected to improve concentrating ability. Despite a reduction of 58% in solute load, no significant increase in concentrating ability occurred. They concluded that the defect in concentrating ability and the occurrence of polyuria were due to an absolute reduction in the reabsorption of solute-free

water in the collecting tubules.

The present results support the view that a situation analogous to osmotic diuresis is present in chronic renal disease. However, the linear relationship between solute excretion and urine flow rate must be governed to a certain extent by tubular reabsorption of water. The extension of the linear relationship to flow rates above 75 ml. per 60 min. in renal disease subjects with polyuria could be explained on the basis of a graded reduction in tubular reabsorption of water.

The results confirmed that polyuria in subjects with renal disease was different from a water diuresis in normal subjects. There were certain similarities between renal disease cases with polyuria and normal subjects undergoing a solute induced diuresis. This suggested that the concentrating ability test could be legitimately applied to renal disease cases with polyuria, provided they had been subjected to the appropriate fluid restriction and any possibility of water diuresis had been excluded.

Concentrating ability in conditions other than chronic renal disease.

Concentrating ability was studied in a group of patients with angio-neurotic oedema. This type of oedema was first recognised by Quinke in 1882, whose name was originally given to the disorder. Since then, many case reports and reviews of this condition have appeared in the literature (Bullock, 1909; Reimann, 1962; Nilson and Floderus, 1964). The oedema is usually recurring, is often periodic, and in some cases familial.

In the present group of subjects with this condition, there was evidence of impaired concentrating ability, but renal function was normal when assessed by urea clearance. None of the patients had any previous history

of renal disease, and no abnormality was detected on examination of the urine, e.g. protein, casts, pus or blood. Investigation of solute excretion in such patients showed that lowered total solute excretion was due mainly to a reduction in electrolyte excretion.

Very few biochemical investigations have been carried out on patients with this condition. In both the present series and those reported in the literature, blood urea, serum electrolytes, albumin, globulin, cholesterol and serum osmolality were all within normal limits.

Reimann (1962) suggested that in the genesis of periodic oedema, some central mechanism was probably involved, e.g. a vasomotor disturbance which was mediated through the autonomic nervous system and which produced responses at different sites in different individuals. Aldosterone is known to be involved in the genesis of most prolonged forms of oedema (Leutschner and Johnson, 1954). One stimulus for aldosterone secretion is a change in the intravascular volume (Bartter, Liddle, Duncan, Barber and Delea, 1956). Originally this stimulus was thought to produce nerve impulses which would affect aldosterone secretion directly. However, it is now known that the primary mechanism for release of aldosterone is independent of the central nervous system.

In 1960, Laragh, Angers, Kelly, and Liebermann showed that an increase in aldosterone secretion was produced by infusion of angiotensin. Angiotensin is produced by the action of renin, a proteolytic enzyme produced by the renal cortex. A reduction in intravascular volume can stimulate secretion of renin. These findings suggest that changes in intravascular volume do not affect aldosterone secretion directly, but are mediated through

this renin-angiotensin system.

In the group of patients studied, the onset of oedema was invariably associated with some period or situation of emotional stress in the life of the patient. It is considered that this could provide a central mechanism which as described above could produce intravascular volume changes and stimulate aldosterone secretion via the renin-angiotensin system. The results showing impaired concentrating ability and reduced electrolyte excretion in such patients suggest that some form of secondary aldosteronism might be associated with this type of oedema.

Abnormally low concentrating ability was not always associated with impaired renal function, as shown in the patients with angioneurotic oedema. Similar results were obtained in a patient with a history of compulsive water drinking. This confirmed the observation in normals that in a post-diuresis period, concentrating ability was impaired.

The observation of abnormally low concentrating ability and urea clearance in a patient with anorexia nervosa illustrated the effect of dietary intake on both tests. The subject had been on a grossly deficient diet for a period of about one year, prior to the investigation. The abnormally low urea clearance was therefore related to the prolonged protein deficiency. In relation to low concentrating ability, it is of interest that Hastings, Gann and Albertsen (1963) observed similar results in obese patients following a 4 - 5 day starvation period. A marked improvement in concentrating ability was produced by administration of glucose to these patients. It is known that the medulla derives most of its energy from anaerobic glycolysis (Horms and Malvin, 1963). In starvation, a limit

will be imposed on the energy available to this region, and consequently the functional activity of this region will be impaired. This would account for the low concentrating ability in the subject with anorexia nervosa.

SUMMARY.

Urine osmolality was studied in normal subjects under different states of hydration, to establish the limits within which it could be used as an index of renal concentrating ability. The aim was to establish a test of renal function based on urine osmolality, which could be applied to subjects with chronic renal disease.

Urine osmolality following 14 - 15 hours dehydration was a relatively poor index of renal concentrating ability in normal subjects. A very wide normal range was obtained. Many of the subjects could achieve a higher urine osmolality throughout the day, without long periods of prior dehydration.

Solute excretion rates were studied in normal subjects at different urine flow rates, and it was found that a linear relationship existed between solute excretion and urine flow rate, in the range 14 - 75 ml. per 60 min. This range of flow rates could be attained under normal hydration or only mild dehydration. At higher urine flow rates, this relationship ceased abruptly, and there was a random scatter of results.

This scatter of results was associated with the onset of a water diuresis. When patterns of solute excretion and urine flow rate were studied during the course of a water diuresis, it was found that the patterns differed, and the peak solute excretion rate occurred 30 - 45 min. before the maximal urine flow rate. In contrast, when diuresis was induced using urea, or Frusemide diuretic, there was no difference between solute excretion and urine flow rate patterns, and both patterns were "in phase" throughout the diuresis. During urea, and Frusemide diuresis, the linear relationship between solute excretion and urine flow rate continued, even at flow rates greater than 75 ml. per 60 min.

The most significant finding in normal subjects was the linear relationship between solute excretion and urine flow rate in the range 14 - 75 ml. per 60 min. The correlation of the data was sufficiently good to allow the use of this relationship as the basis for a test of renal concentrating ability. From the equation relating urine flow rate and solute excretion, the theoretical osmolality corresponding to a given urine flow rate was derived. The ratio observed: theoretical osmolality, expressed as a percentage was taken as an index of renal concentrating ability.

This measure of concentrating ability was applied to normal controls, control patients and patients suffering from various forms of chronic renal disease. Renal disease patients were subdivided into three groups on the basis of their blood urea concentration (normal blood urea, moderately raised, uraemia). To assess the reliability of the test as an index of renal function, urea clearance studies were carried out simultaneously.

In normal controls and control patients, reliable and reproducible results were obtained which compared favourably with urea clearance values. In renal disease patients with normal blood urea concentration, there was a close agreement between the values obtained for both tests. In subjects with moderately raised blood urea, urea clearance values were consistently less than concentrating ability. In uraemic patients, this discrepancy between the two tests was even more marked. Despite the less dramatic deterioration in concentrating ability in the latter two groups, there was a significant correlation between both tests over the entire range of values.

In subjects with polyuria due to renal disease, flow rates of less than 75 ml. per 60 min. could not be attained with the usual amount of fluid restriction. In such subjects, the linear relationship between solute excretion and urine flow rate was apparent even at flow rates greater than 75 ml. per 60 min.

The possible application of the concentrating ability test in other conditions was considered. In a group of patients suffering from angio-neurotic oedema abnormally low concentrating ability was found. The subjects had normal blood chemistry, urea clearance and no signs or previous history of renal disease. The low concentrating ability was associated with abnormally low excretion of electrolyte.

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